Carbohydrate Research, 104 (1982) 309–317
Elsevier Scientific Publishing Company, Amsterdam – Printed in The Netherlands

INVESTIGATIONS ON THE STRUCTURE OF A HEMICELLULOSE FRACTION ISOLATED FROM THE TRUNK OF A YOUNG BAEL (*Aegle marmelos*) TREE

RATAN K. BASAK, PRABIR K. MANDAL, AND AMAL K. MUKHERJEE*
Department of Macromolecules, Indian Association for the Cultivation of Science, Calcutta-7000 32 (India)

(Received December 17th, 1981; accepted for publication, January 12th, 1982)

ABSTRACT

Purified hemicellulose isolated from a young bael (*Aegle marmelos*) tree with 2.5M sodium hydroxide contained D-xylose and 4-*O*-methyl-D-glucuronic acid in the molar ratio of 7.43:1; traces of glucose, galactose, rhamnose, and arabinose were also present. The linkages between the monosaccharide units were determined by methylation analysis of a hemicellulose fraction (II A) and carboxyl-reduced, hemicellulose II A, and the results were corroborated by those from periodate oxidation and Smith degradation. The anomeric configurations of the D-xylopyranosyl residues were determined by chromium(VI) trioxide oxidation of the acetylated, carboxyl-reduced hemicellulose, and the aldobiouronic acid obtained from graded hydrolysis was characterized. These experiments clearly revealed the structure of this hemicellulose.

INTRODUCTION

The structure of the gum surrounding the seeds in bael fruit[1–3] and of the exudate gum[4] of the bael (*Aegle marmelos*) tree have been investigated in detail. Both gums contain (1→3)-linked galactopyranosyl residues in the backbone chains, but different side-chains in these macromolecules. Investigations on the polysaccharides of bael fruit[5] and seeds[5a] were also carried out. It was of interest to study the hemicellulose from the trunks of growing bael trees, and we now report the results of these investigations.

RESULTS AND DISCUSSION

Hemicelluloses I and II were obtained from the trunk of a young bael trees by extraction under a nitrogen atmosphere with M and 2.5M sodium hydroxide solution,

*Present address: Department of Chemistry, Indian Jute Industries Research Association, 17, Taratolla Road, Calcutta-700088, India.

respectively, and precipitation from the extract with ethanol. Hemicellulose I had $[\alpha]_D^{24}$ −37°, and contained xylose (61.8%), 4-*O*-methylglucuronic acid (9.68%), arabinose (1.75%), rhamnose (2.64%), galactose (4.48%), glucose (1.54%), and methoxyl (2.6%), whereas hemicellulose II had $[\alpha]_D^{24}$ −54°, and contained xylose (59.91%), 4-*O*-methylglucuronic acid (9.28%), arabinose (0.8%), rhamnose (1.47%), galactose (3.74%), glucose (2.93%), mannose (0.8%), and methoxyl (4%).

Hemicellulose II was purified by complexing with Fehling solution[6]. The purified material (hemicellulose IIA) had $[\alpha]_D^{24}$ −64.3°, and contained xylose (63.18%), 4-*O*-methylglucuronic acid (9.53%), rhamnose (2.22%), arabinose (1.27%), galactose (3.81%), and glucose (4.44%). The minor sugar components could not be removed by the purification step; however, they could not be detected after methylation of the hemicellulose. Hemicellulose IIA gave a single spot when high-voltage electrophoresis was conducted in phosphate buffer (pH 8), using spraying reagent *3*, indicating that it was homogeneous.

The carboxyl groups in the hemicelluloses (I, II, and IIA) were reduced with 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-*p*-toluenesulfonate[7] (CMC) and sodium borohydride. On hydrolysis, the products yielded xylose and 4-*O*-methylglucose (confirmed by comparison with an authentic sample), together with traces of the other sugars mentioned. This result clearly confirmed the presence of 4-*O*-methylglucuronic acid in all three of the hemicellulose fractions. From the values of specific rotations of the sugars isolated by paper chromatography of the hydrolyzate of the purified hemicellulose, both the xylose (+18°) and the 4-*O*-methylglucopyranuronic acid (+44°) were found to have the D configuration.

In order to ascertain the anomeric configurations of the different sugar residues, the peracetate of carboxyl-reduced hemicellulose IIA was subjected to oxidation with chromium trioxide[8] in acetic acid at 50°, using *myo*-inositol as the internal standard, aliquots being taken at 0, 0.5, and 1 h. After *O*-deacetylation, the alditol acetates were prepared as usual, and the surviving sugars were analyzed by g.l.c. The results (see Table I), showing that both xylose and 4-*O*-methylglucose (formed from 4-*O*-methylglucuronic acid) were degraded rapidly, indicated that they had the β configuration, but characterization of the aldobiouronic acid having a high, positive rotation

TABLE I

SURVIVAL OF SUGARS[a] IN THE OXIDATION OF ACETYLATED, CARBOXYL-REDUCED HEMICELLULOSE IIA WITH CHROMIUM TRIOXIDE

Time (h)	myo-*Inositol*	*Xylose*	4-O-*Methylglucose*
0	50	50.24	11.11
0.5	50	2.18	0.05
1	50	1.80	0

[a]The sugars were analyzed, and estimated, by g.l.c. in column *a* at 190°.

TABLE II

METHYL ETHERS OF SUGARS FROM THE NEUTRAL FRACTION OF THE HYDROLYZATES OF METHYLATED HEMICELLULOSE IIA (A) AND METHYLATED, CARBOXYL-REDUCED HEMICELLULOSE (B)

Sugars[a]	T[b]		*Approximate mol %*		*Mode of linkage*
	a	b	*A*	*B*	
2,3,4-Xyl	0.66	0.57	trace	trace	Xyl*p*-(1→
2,3-Xyl	1.51	1.23	88.6	73	→4)-Xyl*p*-(1→
3-Xyl	2.88	2.15	11.4	14.1	→2,4)-Xyl*p*-(1→
2,3,4,6-Glc	1	1.06	trace	12.1	Glc*p*-(1→

[a]2,3,4-Xyl = 2,3,4-tri-*O*-methyl-D-xylose, etc. [b]Retention times of the corresponding alditol acetates, relative to that of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol as unity, on (*a*) a column of 3% of ECNSS-M at 165°, and (*b*) a column of 3% of OV-225 at 165°.

proved that the glucose derivative had the α configuration. The ready oxidation of the glucose derivative may be due to the presence of a methoxyl group therein. The xylose group, however, had the β configuration, as is also evident from the high negative rotation of the hemicellulose.

The aldobiouronic acid was isolated by hydrolysis of fraction IIA with 0.5M sulfuric acid for 6 h at 100°, and resolution on paper (see Experimental section). On hydrolysis, the acid (R_{Xyl} 0.83, $[\alpha]_D^{24}$ +98.4°) yielded products which, in paper chromatography, had the same mobilities as those of the unhydrolyzed product, 4-*O*-methylglucuronic acid and xylose. Hydrolysis after reduction with sodium borohydride yielded products that showed only a spot of 4-*O*-methylglucuronic acid (and no spot of xylose) when stained with reagent *2*. These results indicated that the aldobiouronic acid was probably 2-*O*-(4-*O*-methylglucopyranosyluronic acid)-D-xylose. To check this, the compound was methylated, and the product was reduced with lithium aluminum hydride in oxolane. Hydrolysis of this product yielded 2,3,4-tri-*O*-methylglucose and 3,4-di-*O*-methylxylose in the ratio of 1.05:1 (see Experimental section). This experiment showed that the aldobiouronic acid was, indeed, 2-*O*-(4-*O*-methylglucopyranosyluronic acid)-D-xylose.

The hemicellulose IIA and carboxyl-reduced hemicellulose IIA were permethylated, first by the Hakomori[9] method and then by the Purdie method[10], until the OH absorption band was absent from the i.r. spectrum. The fully methylated products were hydrolyzed, and the resulting, partially methylated sugars were converted into their alditol acetates, and these analyzed by g.l.c. (see Table II).

As the retention time of 2,3-di-*O*-methylxylose was the same as that of 3,4-di-*O*-methylxylose in both columns tested (1.54 in column *a*, and 1.19 in column *b*), it was not possible to identify the di-*O*-methylxylose by g.l.c. analysis therewith. For the same reason, identification of the mono-*O*-methylxylose was also not possible (for 2-*O*-, 3-*O*-, and 4-*O*-methylxylose, the retention time is 2.92 in column *a*, and 2.15 in column *b*). To overcome these difficulties, a larger amount (350 mg) of

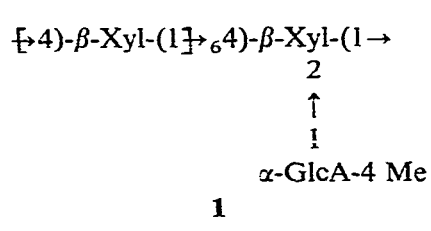
hemicellulose IIA was permethylated and, after hydrolysis, the different methylated sugars were isolated by preparative paper-chromatography. The specific rotations and melting points of the crystalline derivatives revealed the presence of 2,3-di-*O*-methyl- and 3-*O*-methyl-xylose in the hydrolyzate.

Characterization, by g.l.c. analysis, of 2,3,4,6-tetra-*O*-methylglucose in the hydrolyzate of the methylated, carboxyl-reduced hemicellulose showed that 4-*O*-methylglucuronic acid is present in the hemicellulose as nonreducing end-groups. The molar ratios of 2,3-di-*O*-methylxylose, and 3-*O*-methyl- and 2,3,4,6-tetra-*O*-methylglucose were 6.12:1.17:1. These results clearly show that hemicellulose IIA contains chains of (1→4)-linked β-D-xylopyranosyl residues, with approximately every seventh D-xylosyl residue carrying a terminal 4-*O*-methyl-D-glucopyranosyluronic acid group attached as a side chain to O-2 of the D-xylosyl residues.

On reduction and hydrolysis, the acid fraction of the hydrolyzate of methylated hemicellulose IIA yielded 2,3,4-tri-*O*-methylglucose and 3-*O*-methylxylose in almost equal proportions, whereas, if the reduced, methylated aldobiouronic acid (obtained as just described) was further methylated, it yielded 2,3,4,6-tetra-*O*-methylglucose and 3,4-di-*O*-methylxylose in equal proportions (see Experimental section). These results indicated that the acid fraction of the hydrolyzate of the methylated hemicellulose IIA contained a partially methylated aldobiouronic acid whose structure was 3-*O*-methyl-2-*O*-(2,3,4-tri-*O*-methylglucopyranosyluronic acid)xylose.

Hemicellulose IIA was oxidized with 0.1M sodium metaperiodate. Consumption of 0.80 mol of periodate per mol of sugar residue supported the results of the methylation analysis. Smith degradation of the hemicellulose gave glycerol and xylitol in the molar ratio of 6.3:1, with a small proportion of erythritol. The large proportion of glycerol clearly established the presence of (1→4)-linked xylopyranosyl residues in the backbone chain. The small proportion of erythritol must have originated from hexosyl residues, present in the hemicellulose in small proportions.

From the results of these experiments, it is clear that the hemicellulose fraction IIA is essentially a (1→4)-linked β-D-xylan that has a 4-*O*-methyl-α-D-glucopyranosyluronic acid group at certain O-2 atoms. From the analysis of the sugars in the original hemicellulose IIA, and in the methylated hemicellulose IIA, the average repeating-unit of hemicellulose IIA may be assigned as shown in **1**.



1

where Xyl = a D-xylopyranosyl residue, and GlcA-4Me = a 4-*O*-methyl-D-glucopyranosyluronic acid group.

EXPERIMENTAL

General. — All specific rotations are equilibrium values, and were measured with a Perkin–Elmer Model 241 MC spectropolarimeter at 23 $\pm 1°$ and 589.6 nm. All evaporations were conducted *in vacuo* at $\sim 40°$, and small volumes of aqueous solutions were lyophilized. Paper chromatography was performed on Whatman No. 1 and No. 3 papers by the descending technique, using the following solvent systems: (*A*) 8:2:1 ethyl acetate–pyridine–water; (*B*) 9:2:2 ethyl acetate–acetic acid–water; (*C*) 5:5:1:3 ethyl acetate–pyridine–acetic acid–water; (*D*) upper layer of 4:1:5 butanol–ethanol–water; and (*E*) 18:3:1:4 ethyl acetate–acetic acid–formic acid–water. The spray reagents used were: (*1*) alkaline silver nitrate, (*2*) aniline hydrogenoxalate, and (*3*) benzidine periodate. The homogeneity of the hemicellulose was determined by high-voltage electrophoresis on Whatman No. 1 filter paper, using phosphate buffer (pH 8), with a Shandon High-voltage Electrophoresis apparatus Model L-24. Infrared spectra were recorded with a Beckman IR-20A instrument, and ultraviolet and visible spectra with a Yanaco-SPI spectrophotometer. For gas–liquid chromatography, a Hewlett–Packard 5730A gas chromatograph with flame-ionization detector was used. Resolutions were performed in glass columns (1.83 m × 6 mm) containing (*a*) 3% of ECNSS-M on Gas Chrom Q (100–120 mesh), and (*b*) 3% of OV-225 on Gas Chrom Q (80–100 mesh).

Extraction of hemicellulose from the hardwood of a bael (Aegle marmelos) *tree.* — Wood from a growing bael tree was freed of the bark, and then cut into small pieces that were crushed by mechanical hammering, and dried. The crushed material was extracted with 1:2 (v/v) ethanol–benzene to remove fats and coloring matter. The extractive-free wood was dried, and then delignified by passing gaseous chlorine into a suspension of the material in water.

The delignified material (297 g) was extracted with water (1.80 L) for 4 h at 85°. No precipitate was formed on addition of ethanol to the extract. The remaining solid was extracted with M NaOH solution (700 mL) for 2 h at room temperature in an atmosphere of nitrogen, with stirring. The extract was squeezed through a piece of Nylon cloth, and centrifuged for 20 min at 3000 r.p.m. for clarification. The process of extraction was repeated, and the extracts were combined, cooled in an ice-bath, and acidified with glacial acetic acid to pH 4–5. The hemicellulose was precipitated by adding ethanol (3 vol.) with constant stirring. The suspension was centrifuged for 30 min at 6000 r.p.m., and the precipitate washed 3 times with ethanol. The precipitate was dissolved in water (800 mL), reprecipitated with ethanol, washed with dry ethanol, and dried *in vacuo*. The hemicellulose, obtained as a light-brown powder (9 g), had $[\alpha]_D^{24}$ −37° (*c* 1, water).

The material left after the foregoing treatment was extracted with 2.5M NaOH solution (2 × 600 mL), and the hemicellulose was isolated as already described; yield 8.89 g, $[\alpha]_D^{24}$ −54° (*c* 1, water).

The hemicelluloses extracted with M NaOH and 2.5M NaOH solution were respectively named hemicellulose I and II.

Purification of hemicellulose II. — Hemicellulose II was purified by complexation with Fehling solution[6]. Crude hemicellulose II (1 g) was dispersed in water (75 mL), the solution treated with an equal volume of freshly prepared Fehling solution, to precipitate the copper complex, the upper liquid decanted, and the suspension centrifuged. The precipitate was washed with water, and then suspended in water (50 mL), and cold, 0.5M hydrochloric acid was added at 0–5°, with stirring, until the copper complex had been decomposed, giving a slightly opalescent solution from which the hemicellulose was precipitated with an equal volume of acetone. The suspension was centrifuged, and the precipitate was washed with slightly acidified mixtures of acetone and water (3:2, 4:1, 9:1, and 1:0) and then with redistilled ethanol, and dried. The purified material (0.7374 g), $[\alpha]_D^{24}$ −64.28° (*c* 0.84, 0.1M NaOH), was designated IIA.

*Preparation of carboxyl-reduced hemicellulose with 1-cyclohexyl-3(2-morpholinoethyl)carbodiimide metho-*p*-toluenesulfonate (CMC)*[7]. — Hemicellulose IIA (27 mg) was dispersed in water (25 mL), and dissolved by dropwise addition of 0.1M NaOH solution, and the pH brought to neutrality with glacial acetic acid. CMC (680 mg) was added, with stirring, and the pH was kept at 4.75 by addition of 0.01M hydrochloric acid. After 2 h, 2M aqueous sodium borohydride (25 mL) was added during 1 h, and the pH was kept at ~7 by addition of 4M hydrochloric acid. The solution was dialyzed against distilled water for 24 h, and then lyophilized. The whole process was repeated once. Hemicellulose I (27 mg) and hemicellulose II (26 mg) were also reduced in this way.

Hydrolysis, and sugar analysis. — Hemicelluloses I, II, and IIA (10 mg of each) were separately[12] treated with 77% sulfuric acid (0.1 mL) and kept for 30 min at room temperature. The solutions were diluted with water (20 vol.), and then heated for 6 h on a boiling-water bath. The hydrolyzates were made neutral with barium carbonate, and the suspensions centrifuged. Part of each centrifugate was analyzed by paper chromatography using solvent *A*, and *E*. The other part was converted into alditol acetates by reduction with $NaBH_4$ followed by acetylation with acetic anhydride–pyridine, and the alditol acetates were analyzed by g.l.c. in column *a* at 190°. For estimation of sugar residues, a known amount of carboxyl-reduced hemicellulose I, II, and IIA was each mixed with a known amount of *myo*-inositol, and hydrolyzed as before. After the usual treatment, the alditol acetates were prepared, and analyzed by g.l.c. in column *a* at 190°.

Nature of the sugars. — A batch (150 mg) of hemicellulose IIA was hydrolyzed as just described, and the sugars were resolved, and isolated, by preparative paper-chromatography. The specific rotations of these sugars were: xylose, +18° (lit.[13] +19° for D-xylose), and 4-*O*-methylglucuronic acid, +44° (lit.[14] +48° for 4-*O*-methyl-D-glucuronic acid).

Oxidation of the carboxyl-reduced hemicellulose IIA with chromium trioxide[8]. — A mixture of carboxyl-reduced hemicellulose IIA (7.3 mg) and *myo*-inositol (0.9 mg) was dissolved in formamide (1 mL); acetic anhydride (1 mL) and pyridine (1.5 mL) were added, and the mixture was stirred for 16 h at room temperature. The mixture

was evaporated under vacuum, and the residue partitioned between water and chloroform. The chloroform layer was washed with water, dried (anhydrous sodium sulfate), and evaporated to dryness. The whole process was repeated once. The residue was dissolved in glacial acetic acid (3 mL), chromium trioxide (700 mg) was added, and the mixture was stirred on a water bath at 50°. Aliquots were taken at 0, 0.5, and 1 h, immediately diluted with water, and then partitioned between water and chloroform. The chloroform extracts were combined, dried (anhydrous sodium sulfate), evaporated to dryness, the residue deacetylated with 0.2M sodium methoxide, the solution decationized with Dowex 50-W X-8 (H^+) ion-exchange resin, the product hydrolyzed, and the sugars converted into alditol acetates in the usual way. The alditol acetates were analyzed by g.l.c. in column *a* (see Table I).

Methylation analysis. — Hemicellulose IIA (7 mg) and carboxyl-reduced hemicellulose IIA (6 mg) were each dissolved in dry dimethyl sulfoxide (7 and 6 mL, respectively) in separate vials, and then treated with 2M methylsulfinyl sodium (7 and 6 mL, respectively) under nitrogen. The solutions were stirred overnight, and then methyl iodide (7 and 6 mL) was slowly added, with external cooling. The mixtures were stirred for 2 h, dialyzed, and the products extracted with chloroform and water. The chloroform layers were dried (anhydrous sodium sulfate), evaporated to dryness, and the products remethylated by the Purdie method[10]. The fully methylated hemicellulose had no OH bands in its i.r. spectrum. The permethylated hemicellulose IIA (7.2 mg) had $[\alpha]_D^{24}$ −45.8° (*c* 0.72, chloroform), and the permethylated, carboxyl-reduced hemicellulose IIA (6.3 mg) had $[\alpha]_D^{24}$ −11.1° (*c* 0.63, chloroform).

The permethylated samples were hydrolyzed, first with 85% formic acid (3 mL each) for 2 h at 100°, and then, after removal of the formic acid, with 0.5M sulfuric acid for 18 h at 100°. The hydrolyzates were made neutral with $BaCO_3$, and, after the usual treatment, the products were converted into alditol acetates, and these were analyzed by g.l.c. in column *a*, and *b*, at 165° (see Table II).

A batch (340 mg) of hemicellulose IIA was methylated by the Hakomori[9] and the Purdie method[10], and then hydrolyzed as already described. Paper-chromatographic examination of the hydrolyzate (350 mg) revealed the presence of four methylated sugars (solvent *D*, and spray reagent *2*). The mixture was separated on Whatman No. 3 MM paper into its components, and each of them was isolated in the homogeneous state.

Fraction 1. The syrup (65 mg) had $[\alpha]_D^{24}$ +144° and was chromatographically homogeneous; it was reduced with lithium aluminum hydride in oxolane. One part (30 mg) was hydrolyzed with 0.5M sulfuric acid for 20 h, and after the usual treatment, the products were separated by preparative paper-chromatography, using solvent *D* and spraying the guide spots with reagent *2*.

Fraction 1a. The syrup (9 mg) was reduced ($NaBH_4$), and analysis of the alditol acetate by g.l.c. in column *a* at 165° showed the presence of 2,3,4-tri-*O*-methylglucose.

Fraction 1b. Yield 10 mg; it had $[\alpha]_D^{24}$ +17.4° (lit.[15a] +15.5°; lit.[15b] 17.4°). This fraction was characterized as 3-*O*-methylxylose by crystallizing it from methanol–water; mixed m.p. 102–103° (with the sugar from fraction 2), (lit.[15c] m.p. 95–97°).

The other part (30 mg) of the reduced ($LiAlH_4$ in oxolane) material from fraction 1 was methylated by the Kuhn method[17], and the product hydrolyzed. A small amount of the hydrolyzate material was analyzed as its alditol acetate by g.l.c. (column *a* at 165°), which gave peaks of 2,3,4,6-tetra-*O*-methylglucose and 3,4(and/or 2,3)-di-*O*-methylxylose in almost equimolar proportions. The rest of the hydrolyzate was resolved on paper, and the di-*O*-methyl fraction ($[\alpha]_D^{24}$ +18° in water) was identified as 3,4-di-*O*-methylxylose by preparing its crystalline anilide, m.p. 115°.

Fraction 2. Yield 50.7 mg. The identity of this fraction as 3-*O*-methylxylose was confirmed by crystallizing it from methanol–water; m.p. 102–103° (lit.[15c] 95–97°) and $[\alpha]_D^{24}$ +18.8° (*c* 0.19, water); lit.[15a] +15.5°; lit.[15b] 17.4°.

Fraction 3. Yield 1.963 g. The crystalline material had $[\alpha]_D^{24}$ +22.2° (*c* 0.18, water) (lit.[15a] +25°). The syrup (25 mg) was refluxed with freshly distilled, ethanolic aniline (5 mL, containing 1.25 mL of aniline) for 6 h, and the solution was kept overnight at room temperature, evaporated under vacuum at 50°, and the product, *N*-phenyl-2,3-di-*O*-methyl-D-xylosylamine, crystallized from ethyl acetate–pet. ether, m.p. 124° (lit.[15a] 121°).

Fraction 4. The syrup (15 mg) had $[\alpha]_D^{24}$ +15.3° (*c* 0.15, water), lit.[15b] +18.5°. On reduction ($NaBH_4$), and analysis of the alditol acetate by g.l.c. in column *a* at 165°, it showed the peak for 2,3,4-tri-*O*-methylxylose.

Periodate oxidation and Smith degradation[11] of hemicellulose IIA. — Hemicelluiose IIA was treated with 0.1M sodium metaperiodate in the dark at 10°. Uptake of periodate (monitored spectrophotometrically[16]) became constant at 196 h, and corresponded to a consumption of 0.80 mol of the oxidant per mol of sugar residue.

In a separate experiment, hemicellulose IIA (9.5 mg) was treated with 0.1M sodium metaperiodate (10 mL) in the dark for 196 h at 10°. The excess of periodate was decomposed by adding ethylene glycol (4 mL) and keeping the mixture for 3 h. The solution was dialyzed, and then concentrated to 3 mL. The concentrate was treated with sodium borohydride (60 mg), kept overnight at room temperature, acidified with acetic acid, dialyzed, evaporated, and the product hydrolyzed. After the usual treatment, the hydrolyzate was converted into alditol acetates, and these were analyzed by g.l.c. in column *a* at 190°.

Characterization of the aldobiouronic acid. — Hemicellulose IIA (2 g) was hydrolyzed with 0.5M sulfuric acid (130 mL) for 6 h at 100°. The solution was cooled, made neutral with barium carbonate, the suspension filtered, the filtrate concentrated to a small volume, and the concentrate poured into ethanol. The precipitated barium salts were extracted with boiling ethanol to remove xylose. The barium salts were dissolved in water, and the solution was treated with Amberlite IR-120 (H^+) resin, to remove barium ions, and evaporated to a syrup. Paper chromatography (solvent *B*) of the syrup showed spots for monosaccharides and spots in the region of oligosaccharides. The oligosaccharide having R_x 0.83 was found in large proportion, and was isolated in a homogeneous state by resolving on a preparative paper-chromatogram.

The oligosaccharide had $[\alpha]_D^{24}$ +98.5°, and, on hydrolysis and paper-chromato-

graphic examination, showed spots of xylose, 4-*O*-methylglucuronic acid, and the original compound, whereas, on reduction ($NaBH_4$) and hydrolysis, it showed no spot of xylose. The oligomer was methylated by the Kuhn method[17]. Part of the methylated product was hydrolyzed, and analysis of the alditol acetate by g.l.c. in column *a*, and *b*, showed a peak for 3,4-(and/or 2,3)-di-*O*-methylxylose only. Another part of the methylated material was reduced with lithium aluminum hydride in oxolane. After hydrolysis of the reduction product, the material was analyzed by g.l.c. in column *a*; it showed peaks for 3,4-(and/or 2,3)-di-*O*-methylxylose and 2,3,4-tri-*O*-methylglucose in equal proportions (1.05:1). The di-*O*-methylxylose was isolated on paper, and the structure was confirmed as being 3,4-di-*O*-methylxylose (as with the methylated aldobiouronic acid reported earlier).

REFERENCES

1 A. Roy, A. K. Mukherjee, and C. V. N. Rao, *Carbohydr. Res.*, 41 (1975) 219–226.
2 A. Roy, S. B. Bhattacharya, A. K. Mukherjee, and C. V. N. Rao, *Carbohydr. Res.*, 50 (1976) 87–96.
3 A. Roy, A. K. Mukherjee, and C. V. N. Rao, *Carbohydr. Res.*, 54 (1977) 115–124.
4 P. K. Mandal and A. K. Mukherjee, *Carbohydr. Res.*, 84 (1980) 147–159.
5 R. K. Basak, P. K. Mandal, and A. K. Mukherjee, *Carbohydr. Res.*, 97 (1981) 315–321.
5a P. K. Mandal and A. K. Mukherjee, *Carbohydr. Res.*, 90 (1981) 233–241.
6 S. K. Chanda, E. L. Hirst, J. K. N. Jones, and E. G. V. Percival, *J. Chem. Soc.*, (1950) 1289–1297.
7 R. L. Taylor and H. E. Conrad, *Biochemistry*, 11 (1972) 1383–1388.
8 J. Hoffman, B. Lindberg, and S. Svensson, *Acta Chem. Scand.*, 26 (1972) 661–666.
9 S.-I. Hakomori, *J. Biochem. (Tokyo)*, 55 (1964) 205–208.
10 T. Purdie and J. C. Irvine, *J. Chem. Soc.*, 85 (1904) 1049–1070.
11 M. Abdel-Akher, J. K. Hamilton, R. Montgomery, and F. Smith, *J. Am. Chem. Soc.*, 74 (1952) 4970–4971.
12 J. E. Jeffery, E. V. Partlow, and W. J. Polglase, *Anal. Chem.*, 32 (1960) 1774–1777.
13 I. Heilbron and E. M. Bunbury, *Dictionary of Organic Compounds*, Vol. 4, Eyre and Spottiswoode, London, p. 685.
14 H. C. Srivastava and G. A. Adams, *J. Am. Chem. Soc.*, 81 (1959) 2409–2412.
15 (a) U. Sharma and A. K. Mukherjee, *Carbohydr. Res.*, 95 (1981) 81–86. (b) P. C. Das Gupta and P. P. Mukherjee, *J. Chem. Soc., C*, (1967) 1179–1182. (c) H. O. Bouveng, *Acta Chem. Scand.*, 15 (1961) 87–95.
16 J. S. Dixon and D. Lipkin, *Anal. Chem.*, 26 (1954) 1992–1993.
17 R. Kuhn, H. Trischmann, and I. Löw, *Angew. Chem.*, 67 (1955) 32–37.