mM, or 17 moles for the base mol. wt. of 3460 assumed for fraction R. Other 5-ml. aliquots were back-titrated to the original pH of 4.89 by 0.01 N alkali, and the volumes added were 0.85, 0.85, 0.85, 1.18 and 1.35 ml. after 3.4, 4.2, 5.3, 8.4 and 26 hr., respectively. These figures corresponded to the production of 1.04 moles of formic acid per base mol. wt. of 3460 in 3 to 5 hr. and to 1.6 moles after 26 hr.

Methylation of Fraction R (Table IV).—The sample, 8.7 g., was stirred under nitrogen for 2 hr. with 140 ml. of N aqueous thallous hydroxide,[37] and after recovery the insoluble, yellow thallium derivative was thoroughly dried and finely powdered; yield 27.2 g. This product was methylated for 12 hr. with methyl iodide, but more than half was almost unchanged. The unchanged portion was remethylated in the same way and then combined with the remainder. The whole, OCH_3, 31%, was then methylated by thallous ethylate in benzene followed by methyl iodide and by silver oxide–methyl iodide to a constant methoxyl content of 36.8%; yield 8.5 g. or 97% by weight.

After being fractionated (Table V) the sub-fractions were recombined and 6.4 g. was degraded by being boiled for a total of 10 hr. with 330 ml. of 2% methanolic hydrogen chloride. Water, 300 ml., was added and boiling continued for a further 6 hr. to hydrolyze esters and glucosides. After removal of hydrogen chloride (as the silver salt) and of the solvent, the residual light yellow sirup, 6.1 g., was dissolved in 7 ml. of methyl ethyl ketone saturated with water and was applied to the top of a column of powdered cellulose, 3.7 cm. by 51 cm., previously washed with the solvent. More solvent was percolated through the column at the rate of 6 ml. in 5 min., and the progress of the separation was followed by paper chromatography. Eluates 16 to 56 contained dimethylxylose, and eluates 59 to 100 monomethylxylose, but both fractions also contained traces of uronic acids. These traces were removed by shaking aqueous solutions of the fractions with a mixture of Amberlite resins IR-120 and IR-4B, the filtrates being separately evaporated to sirups.

The dimethylxylose sirup, 2.87 g., had a specific dextrorotation of 23.8° in water (c 2.0), and when chromatographed with the methyl ethyl ketone, water–ethanol developer gave a single spot with R_f 0.65.

Anal. Calcd. for $C_5H_8O_3(OCH_3)_2$: OCH_3, 34.8. Found: OCH_3, 34.4, 34.6

The m.p. of the anilide, 124–125°, was undepressed by admixture with an authentic sample of 2,3-di-*O*-methylxylose anilide kindly supplied by Dr. G. A. Adams, of the National Research Council of Canada

The monomethylxylose sirup, 0.20 g., with a specific dextrorotation of 23° in water (c 1.0), gave a principal spot with R_f 0.43, and a slight spot with R_f 0.65 when chromatographed for 2.5 hr. The latter spot corresponded to dimethylxylose.

Anal. Calcd. for $C_5H_9O_4(OCH_3)$: OCH_3, 18.9. Found: OCH_3, 19.9, 19.8.

After 12.5 hr. the principal spot had nearly divided into two spots with centers 36.3 and 38.3 cm. from the starting line. These spots were identical in position to those pertaining to 2- and 3-*O*-methylxylose, respectively. The reduction of 200 ml. of 0.05 N lead tetraacetate in acetic acid containing 87 mg. of the original sirup was followed iodometrically[38] and within two minutes amounted to 0.52 mole per mole. This proportion of the sirup therefore consisted of 3-*O*-methylxylose. The reduction did not increase within 15 min.

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MONTREAL, CANADA

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

The Constitution of the Hemicellulose of Western Hemlock (*Tsuga heterophylla*). II. Hydrolysis of the Methylated Hemicellulose[1,2]

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The hemicellulose isolated from delignified Western Hemlock wood, by extraction with alkali, has been shown to contain a branched chain arabo-methoxyglucurono-xylan. The side chains consist of single units of 4-*O*-methyl-D-glucuronic acid and single units of L-arabofuranose joined to positions 2 and 3, respectively, of D-xylopyranose units of the xylan molecular framework. The methylated hemicellulose gives upon hydrolysis: 2,3,4-tri-*O*-methyl-D-xylose (1 mole), 2,3,5-tri-*O*-methyl-L-arabinose (0.75 mole), 2,3-di-*O*-methyl-D-xylose (8 moles), 2-*O*-methyl-D-xylose (1 mole), 3-*O*-methyl-D-xylose (3 moles) and 2,3,4-tri-*O*-methyl-D-glucuronic acid (3 moles). The general structural features of the polysaccharide are discussed.

An aldobiouronic acid, 2-*O*-(4-*O*-methyl-D-glucuronosyl-D-xylose, has been shown[3] to be a component of the hemicellulose of Western Hemlock (*Tsuga heterophylla*). This paper is concerned with the main structural features of the hemicellulose itself as revealed by methylation studies.

In order to ascertain the mode of union of the building units of the hemicellulose the latter was methylated first with potassium hydroxide and methyl sulfate[4] and then with silver oxide and methyl iodide.[5] Fractional precipitation of the methylated polysaccharide from chloroform with petroleum ether indicated the presence of two principal components. The less soluble methylated polysaccharide which had $[\alpha]^{25}D$ −51° (chloroform) is the subject of the present paper. The more soluble methylated polysaccharide showing $[\alpha]^{25}D$ −13° (chloroform) will be discussed in a subsequent communication.

Following methanolysis of the methylated polysaccharide with 2% methanolic hydrogen chloride under conditions which left the sugar acid in the form of a disaccharide, the methyl glycosides were separated into neutral and acidic components using ion exchange resins.

The acidic component was shown by analysis to be a partially methylated aldobiouronic acid. This was identified as methyl 2-(2,3,4-tri-*O*-methyl-D-

(1) Paper No. 3488, Scientific Journal Series, Minnesota Agricultural Experiment Station.

(2) This paper was presented at the 128th A.C.S. Meeting in Minneapolis, Minn., September, 1955.

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glucuronosyl)-3-*O*-methyl-D-xyloside by lithium aluminum hydride reduction[6,7] of the corresponding methyl ester to give a neutral disaccharide. A portion of this neutral methylated disaccharide crystallized spontaneously (m.p. 165–167°) and since it had $[\alpha]^{27}D$ +79° (water), it appeared to be the α-anomer I. The remaining sirupy component having $[\alpha]^{27}D$ +96° (water) contained the β-form. When the neutral methylated disaccharide was hydrolyzed and the two components separated on a cellulose–hydrocellulose column,[8] there were obtained 2,3,4-tri-*O*-methyl-D-glucose, identified as its characteristic crystalline anilide,[9] and 3-*O*-methyl-D-xylose identified both as the crystalline sugar[10] and as its crystalline anilide.[10] These results are in agreement with those found for the fully methylated disaccharide derived from the aldobiouronic acid.[3]

The mixture of the neutral sugar glycosides obtained from the methanolysis of the methylated Western Hemlock hemicellulose was hydrolyzed and resolved into its components on a cellulose–hydrocellulose column.[8] The mixture was found to contain 2,3,4-tri-*O*-methyl-D-xylose, 2,3,5-tri-*O*-methyl-L-arabinose, 2,3-di-*O*-methyl-D-xylose, 2-*O*-methyl-D-xylose and 3-*O*-methyl-D-xylose.

The 2,3,4-tri-*O*-methyl-D-xylose was identified by qualitative chromatography, by rotation and as the crystalline 2,3,4-tri-*O*-methyl-D-xylono-δ-lactone.[11] The 2,3,5-tri-*O*-methyl-L-arabinose was identified after bromine oxidation as 2,3,5-tri-*O*-methyl-L-arabonamide.[12] The 2,3-di-*O*-methyl-D-xylose was characterized by conversion into its crystalline anilide,[13] while the 2- and 3-*O*-methyl derivatives of D-xylose were identified as the crystalline sugars.[10,14]

From the above experimental evidence, certain structural features of the hemicellulose can be deduced. It is clear that the 2,3,4-tri-*O*-methyl-D-xylose is derived from terminal D-xylopyranose units in the polysaccharide, and from the large amount of 2,3-di-*O*-methyl-D-xylose obtained it is concluded that the main body of the polysaccharide consists of D-xylose units of the pyranose type linked through positions 1 and 4. The 2-*O*-methyl-D-xylose is derived from units of xylose which form branch points in the molecule; clearly these units are joined through position 3 in addition to positions 1 and 4. Likewise the 3-*O*-methyl-D-xylose represents a branch point with linkages at positions 1, 2 and 4.

Considering the mole ratios of the cleavage products of the methylated hemicellulose (see Table I), it will be apparent that the polysaccharide molecule has three aldobiouronic acid residues associated with about 13 D-xylose units. The characterization of the disaccharide I proves that the three 4-*O*-methyl-D-glucuronic acid residues are linked as single unit side chains to position 2 of three D-xylose units of the main chain. It also appears that one L-arabofuranose residue is linked as a side chain to a D-xylose unit at position 3. In stating this it is assumed that the figure of 0.75 (Table I) for 2,3,5-tri-*O*-methyl-L-arabinose is low due to volatility and that a value of 1.0 mole is more likely in view of the isolation of 1.0 mole of 2-*O*-methyl-D-xylose.

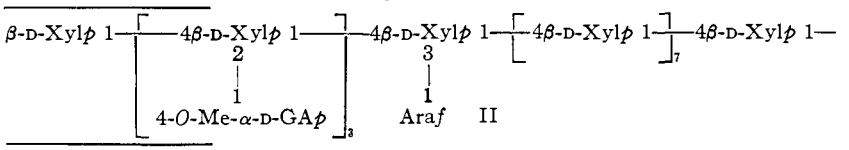
Although the trace amounts of D-xylose isolated from the hydrolyzate of the methylated polysaccharide could conceivably arise from demethylation[15] or incomplete methylation,[16] the possibility should not be excluded that it represents a unit in the molecule at which multiple branching occurs.

TABLE I

THE HYDROLYSIS PRODUCTS OF POLYSACCHARIDE A FROM METHYLATED WESTERN HEMLOCK HEMICELLULOSE

Sugar derivative	Wt., mg.	Mole ratio (approx.)
2,3,5-Tri-*O*-methyl-L-arabinose	76	0.75
2,3,4-Tri-*O*-methyl-D-xylose	104	1
2,3-Di-*O*-methyl-D-xylose	768	8
3-*O*-Methyl-D-xylose	267	3
2-*O*-Methyl-D-xylose	88	1
2,3,4-Tri-*O*-methyl-D-glucuronic acid	348	3
D-Xylose	12 (approx.)	Trace

From these facts a simplified structure II for Western Hemlock hemicellulose may be tentatively proposed.



For convenience the molecule is written with a free reducing group. This may or may not be the case, for it is conceivable that the structure depicted may be joined to another one and thus may provide a branching xylose unit which would give rise to the formation of the 2-*O*-methyl-D-xylose upon methylation.

This additional information concerning the hemicellulose of Western Hemlock, together with that already known about the hemicellulose of esparto grass,[17,18] wheat straw,[19,20] corn cobs,[20] etc., em-

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phasizes the view that in all these polysaccharides there is present a linear framework of D-xylopyranose units. That the properties of these hemicelluloses vary considerably would now seem to be due, in part at least, to the variation in the nature and the length of the side chains attached to the main structural framework of D-xylopyranose units. The view has been expressed[21] that an increase in the number of L-arabofuranose units increases the solubility of the hemicellulose, and it seems probable that an increase in the number of uronic acid residues has a similar effect. Although there are a relatively large number of side chains in the Western Hemlock hemicellulose, namely, three of 4-*O*-methyl-D-glucuronic acid and one of L-arabinose for every twelve D-xylose units of the main chain, linear character is probably a dominant feature of the molecular structure of the hemicellulose. This linear type of structure, which is probably common to the hemicelluloses of other wood holocelluloses, may be one of the major factors responsible for the very close association between the hemicelluloses and cellulose. The only hard wood hemicelluloses whose structures have been studied in detail are those of beech[22] and birch.[23] In the former case the hemicellulose consists of a β-1,4-linked xylan having a glucuronic acid residue attached to position 2 of one out of every 70 xylose units in the main chain. In the case of birch hemicellulose similar results were obtained except that the chain length was considered to be 22 units. The present paper represents the first detailed study of a hemicellulose from a coniferous wood and, although the structural features conform to the general pattern of a β-1,4-linked xylan, the hemicellulose of Western Hemlock is distinguished by the presence of a high proportion of uronic acid residues.

Experimental[24]

Isolation of Western Hemlock Hemicellulose.—The hemicellulose was isolated from chlorite holocellulose by extraction with alkali and purified through its copper complex as previously described.[3]

Attempted Fractionation of Hemicellulose.—A portion of the hemicellulose was dissolved in cuprammonium solution and fractionally precipitated by the addition of 5% sodium hydroxide according to the method of Hess and Lüdtke.[25] Several fractions were obtained varying in neutralization equivalent from 1270 to 950, but no clear cut fractionation could be achieved.

Acetylation of Hemicellulose.—A solution of the hemicellulose (1.5 g.) in formamide (30 ml.) was treated with pyridine (30 ml.) followed by acetic anhydride (5 ml.) which was added slowly with shaking. No heat was evolved, and after 1 hr. a further 5 ml. of acetic anhydride was added. After standing overnight a third portion of acetic anhydride was added, and 24 hr. later the brown solution was poured into an excess of 1% hydrochloric acid. The solution was stirred periodically for several hours in order to neutralize the pyridine and the solid collected by centrifugation. Washing with alcohol and ether and air drying yielded the acetate as an almost white solid (2.4 g. having $[\alpha]^{23}D$ −28.4° (*c* 2.3 in chloroform). Attempted fractionation from chloroform with petroleum ether yielded a range of fractions varying in rotation from $[\alpha]^{23}D$ −47.0° to −10.8° (*c* 1 in chloroform). No well-defined break was observed and hydrolysis of the deacetylated fractions showed the presence of xylose and mannose in all fractions.

Methylation of Western Hemlock Hemicellulose.—Hemicellulose (15 g.) was dissolved in water (150 ml.) by mechanical shaking for 4 hr. To the clear yellow solution potassium hydroxide (150 g.) was added with cooling. The polysaccharide remained in solution and was methylated at 50° by the dropwise addition of methyl sulfate (100 ml.) over a period of 3 hr. After the first hour, aqueous potassium hydroxide (150 ml., 45%) was added simultaneously. Small amounts of acetone were added periodically to control foaming and to reduce viscosity. When all of the reagents had been added, stirring was stopped and the flask heated in a boiling water-bath for 1 hr. to destroy the excess of the methyl sulfate and to distil off the acetone. The partly methylated polysaccharide did not separate satisfactorily, hence the cooled solution was dialyzed overnight against tap water, and the residual solution was evaporated to small bulk.

A second methylation was performed at 50°, using aqueous potassium hydroxide (375 ml., 45%) and methyl sulfate (125 ml.), together with acetone as necessary. The product was recovered by dialysis as before and four further methylations were carried out.

After the sixth methylation the aqueous solution was acidified with hydrochloric acid and extracted with chloroform. The chloroform layer was washed with water until free from chloride ion and evaporated to a sirup which was dried by distillation with successive small portions of 1,4-dioxane. The resulting thick brown sirup was dissolved in chloroform (200 ml.), centrifuged from a small amount of inorganic matter and precipitated by pouring into petroleum ether (6 liters) with mechanical stirring. The curdy solid was washed with petroleum ether and dried *in vacuo* at 25°; yield 11.75 g., $[\alpha]^{27}D$ −29° (*c* 1.2 in chloroform).

A portion (9 g.) of the partly methylated material was dissolved in methyl iodide (100 ml.) and acetone (50 ml.), silver oxide (25 g.) being added in five portions over 20 hours to the refluxing solution.[5] The excess of the methyl iodide was recovered by distillation and the insoluble residue was repeatedly extracted with boiling acetone. Evaporation of the combined acetone extracts gave a sirup which was soluble in methyl iodide, and it was therefore remethylated by Purdie's procedure without the addition of acetone. A third methylation was performed in a similar manner. A small sample of the final sirup, dissolved in chloroform and poured into an excess of petroleum ether, gave an almost white powder, $[\alpha]^{27}D$ −30.5° (*c* 2 in chloroform); OMe, 40.8.

Fractionation of the Methylated Hemicellulose.—The sirup obtained from the third Purdie methylation was dissolved in chloroform (100 ml.), diluted with diethyl ether (100 ml.) and fractionated by the addition of petroleum ether (30–60°) to a mechanically stirred solution. The results are shown in Table II. Fractions 4 and 5 were reprecipitated as shown in Table III. From these results it was concluded that the methylated hemicellulose contained at least two components, one (polysaccharide A) having $[\alpha]^{25}D$ −51° (*c* 2 in chloroform), equiv. wt. 1220, and the other (polysaccharide B) having $[\alpha]^{25}D$ −13°

TABLE II

FRACTIONAL PRECIPITATION OF METHYLATED WESTERN HEMLOCK HEMICELLULOSE

Fraction	Total petroleum ether added, ml.	Weight,[a] g.	$[\alpha]^{25}D$ ($CHCl_3$) (*c* 2)	OMe, %
1[b]	120	0.56	.....	..
2	160	1.97	−51.0°	39.7
3	210	0.85	−51.2	39.6
4	360	2.20	−20.5	41.5
5	[c]	2.15	−10.3	42.0

[a] Fractions 2–4 were precipitated as oils which were dissolved in chloroform (30 ml.) and precipitated as flocculent solids by pouring into petroleum ether (500 ml.). [b] This fraction contained inorganic material and was not examined further. [c] Recovered from mother liquor of fraction 4 by concentration to small volume and precipitation with excess petroleum ether.

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(24) All evaporations were carried out under reduced pressure and at a bath temperature not exceeding 40°.

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TABLE III

REFRACTIONATION OF FRACTIONS 4 AND 5

Fraction	Total petroleum ether added, ml.	Weight,[a] g.	$[\alpha]^{25}D$ ($CHCl_3$) (c 2)	OMe, %
4_1	125	0.22	−35.9°	..
4_2	200	1.35	−13.5	42.0
4_3	300	0.25	− 8.3	..
5_1	300	0.19	− 8.0	..
5_2	900	1.12	−12.9	42.7
5_3	[b]	0.24	−13.3	..

[a] All fractions (except 5_3) were reprecipitated (see note [a], Table II). [b] Recovered from mother liquor (see note [c], Table II).

(c in chloroform), equiv. wt. 1460. The following experimental data refer only to polysaccharide A. Polysaccharide B will form the subject of a later communication.

Methanolysis of Polysaccharide A.—Polysaccharide A (2.12 g.) was dissolved in methanol (50 ml.) containing hydrogen chloride (3%) and refluxed for 10 hr., when the rotation was constant. The solution was neutralized with silver carbonate, filtered and evaporated to a thick sirup (2.45 g.) of the methyl glycosides.

The sirup was dissolved in barium hydroxide solution (25 ml., saturated at room temperature) and heated for 2 hr. at 60° to saponify the methyl ester of the acidic component. The cooled aqueous solution was then continuously extracted with petroleum ether (150 ml.) for 20 hr. The aqueous solution was filtered from traces of solid, passed through a column of Amberlite IR 120 resin and the resin washed with water until the effluent gave a negative Molisch test.

Separation of the Acidic Component of Polysaccharide A.—The effluent from the cation exchange resin was passed through a column of Duolite A4 resin which selectively removed the acidic component. The column was washed with water until the washings gave a negative Molisch test and the effluent and washings were evaporated to a sirup. The petroleum ether extract was added to this sirup and the whole re-evaporated to constant weight; yield of methyl glycosides, 1.52 g.

The acidic component was isolated from the column by displacement with *N* sodium hydroxide solution (25 ml.) and the yellow eluate passed through a fresh column of Amberlite IR 120 to remove the alkali. Evaporation of the effluent and washings furnished the acidic component as a hygroscopic glassy solid $[\alpha]^{25}D$ +88° (c 2.3 in methanol) (yield 0.597 g.). Analysis showed this solid to be principally a partially methylated aldobiouronic acid.

Anal. Calcd. for $C_{16}H_{28}O_{11}$: OMe, 39.2; equiv. wt., 396. Found: OMe, 37.1; equiv. wt., 340.

Preparation of Methyl 2-*O*-(2,3,4-Tri-*O*-methyl-D-glucuronosyl)-3-*O*-methyl-D-xyloside Methyl Ester.—The aldobiouronic acid (566 mg.) was dissolved in methanol (25 ml.) and treated with ethereal diazomethane until the yellow color persisted. After 10 minutes at room temperature the solvent and excess diazomethane were removed by distillation, yielding a pale brown sirup. Purification by extraction with ether (50 ml.) gave the methyl ester of methyl 2-*O*-(2,3,4-tri-*O*-methyl-D-glucuronosyl)-3-*O*-methyl-D-xyloside as a thick sirup (506 mg.) which gave a strong positive hydroxamic acid test for an ester.

Reduction of the Methyl Ester of Methyl 2-*O*-(2,3,4-Tri-*O*-methyl-D-glucuronosyl)-3-*O*-methyl-D-xyloside.—The ester (486 mg.) was dissolved in ether (20 ml., dried by distillation from lithium aluminum hydride). A solution of lithium aluminum hydride (0.5 g.) was prepared by adding the finely crushed hydride to dry ether (20 ml.) and refluxing for 1 hr., by which time the majority of the solid had dissolved. The solution of the ester was added gradually with swirling to the hydride solution at room temperature. When the addition was complete the solution was refluxed for 1 hr. Excess hydride was decomposed by addition of ethereal ethyl acetate followed by dilute aqueous acetic acid until the solution was acidic. Without attempting to separate the reduced material, the whole was evaporated to dryness and anhydrous sodium acetate (0.5 g.) and acetic anhydride (15 ml.) were added. Simultaneous cleavage of the inorganic complex and acetylation of the disaccharide was achieved by heating the mixture on the water-bath for 3 hours. The excess acetic anhydride was removed by distillation under reduced pressure and dilute hydrochloric added to dissolve the inorganic salts. The aqueous solution was extracted four times with chloroform (125 ml., total), the chloroform layer washed with water until free from chloride ion and evaporated to a thick sirup (yield 546 mg.).

Deacetylation was effected by dissolving the sirup in ethanol (10 ml.), adding *N* sodium hydroxide (6 ml.) and heating under reflux for 1 hr. on the steam-bath. Deionization and removal of unreduced aldobiouronic acid was effected by passing the solution successively through Amberlite IR 120 and Duolite A4 resins. Evaporation of the effluent gave an almost colorless sirup (410 mg., yield 91%). Unreduced aldobiouronic acid was recovered by elution with *N* sodium hydroxide (5 ml.) and by passing the alkaline eluate through Amberlite IR 120 resin. Evaporation of the effluent yielded a small amount of dark brown sirup (30 mg.) which had a strongly acid reaction and gave a positive Molisch reaction.

On standing overnight the neutral sirup partly crystallized. Removal of the sirup and washing the crystals with ethyl acetate followed by recrystallization from the same solvent yielded large crystals (50 mg. approx.) of methyl 2-*O*-(2,3,4-tri-*O*-methyl-D-glucopyranosyl)-3-*O*-methyl-β-D-xyloside, m.p. 165–167°, $[\alpha]^{27}D$ +79° (c 0.5 in water).

Anal. Calcd. for $C_{16}H_{30}O_{10}$: OMe, 40.6. Found: OMe, 40.6. The residual sirup had $[\alpha]^{27}D$ +96° (c 1.3 in water) and OMe, 39.4.

Hydrolysis of Methyl 2-O-(2,3,4-Tri-*O*-methyl-D-glucopyranosyl)-3-*O*-methyl-D-xyloside.—The sirupy disaccharide (240 mg.) obtained above was dissolved in methanol (10 ml.) containing 3% hydrogen chloride. When the solution was refluxed, the rotation changed from $[\alpha]^{27}D$ +95° to $[\alpha]^{27}D$ +66° (5 hr., constant). Neutralization (Ag_2CO_3), filtration and evaporation gave a product which was purified by extraction with ethyl acetate (yield of pale yellow sirup, 250 mg.).

When a solution of the mixture of glycosides (250 mg.) in *N* sulfuric acid (5 ml.) was heated on the steam-bath, the rotation changed as follows: $[\alpha]^{28}D$ +63° (initial), +52° (2 hr.), +48° (4 hr.), +46° (6 hr., constant). The calculated rotation for an equimolecular mixture of 3-*O*-methyl-D-xylose, $[\alpha]D$ +17°, and 2,3,4-tri-*O*-methyl-D-glucose, $[\alpha]D$ +67°, is $[\alpha]D$ +42°. Removal of the acid by passage through a column of Duolite A4 resin and concentration of the effluent yielded a sirup (210 mg.). A portion of this sirup, when chromatographed on a paper, showed two spots, corresponding to a trimethylglucose and a monomethylxylose. The remainder of the sirup was added to a cellulose–hydrocellulose column[8] and eluted with methyl ethyl ketone–water azeotrope.[26] The eluate (12 ml. per fraction) was collected at 20-minute intervals 5 hr. after the addition of the sirup. It was found that the faster component was contained in tubes 15–28 and the slower component in tubes 75–95.

Identification of 2,3,4-Tri-*O*-methyl-D-glucose.—Concentration of the contents of tubes 15–28 gave a sirup (63 mg.) which was shown to be chromatographically identical with 2,3,4-tri-*O*-methyl-D-glucose. The anilide had m.p. and mixed m.p. 144–147° on recrystallization from ethyl acetate–petroleum ether. The literature[9] quotes 145–146°.

Identification of 3-*O*-Methyl-D-xylose.—Nucleation of the sirup obtained from the contents of tubes 75–95 gave feathery crystals of 3-*O*-methyl-D-xylose, m.p. and mixed m.p. 97–99°, after recrystallization from acetone.[10] The anilide had m.p. 136–137°, after recrystallization from ethyl acetate.

Separation of the Neutral Components of Methylated Western Hemlock Hemicellulose.—The sirup (1.52 g.) containing the methyl glycosides of the methylated sugars was dissolved in *N* sulfuric acid (60 ml.) and heated for 7 hr. on a steam-bath when the rotation was constant. The solution was neutralized ($BaCO_3$), filtered and the residue extracted with aqueous methanol. Concentration of the filtrate and washings yielded a light brown sirup (1.32 g.).

A mixture of methylated sugars (1.25 g.) was dissolved in a small amount of methyl ethyl ketone–water azeotrope and placed on a column (40 × 3 cm.) packed with a mixture

(26) L. Boggs, L. S. Cuendet, I. Ehrenthal, R. Koch and F. Smith, *Nature*, **166**, 520 (1950).

of hydrocellulose and cellulose (1:1).[8] The same solvent was used for elution and the effluent (18 ml. per fraction) was collected in test-tubes which were changed every 30 minutes. The distribution of the sugars was determined by placing five drops of the contents of each tube on paper and spraying with *p*-anisidine trichloroacetate.[27] The results are shown in Table IV.

TABLE IV

SEPARATION OF NEUTRAL SUGARS OF POLYSACCHARIDE A ON A CELLULOSE–HYDROCELLULOSE COLUMN

Tube number	Component number	Identity of component
5–9	1	2,3,4-Tri-*O*-methyl-D-xylose and 2,3,5-Tri-*O*-methyl-L-arabinose
15–34	2	2,3-Di-*O*-methyl-D-xylose
87–105	3	3-*O*-Methyl-D-xylose
106–120	3 + 4	
121–140	4	2-*O*-Methyl-D-xylose
176–250	5	D-Xylose

The various components were obtained by concentration of the appropriate eluates and the resulting sirups were dissolved in a small amount of acetone (methanol for component 5), filtered and evaporated to constant weight.

Identification of the Components. (a) The Isolation of 2,3,5-Tri-*O*-methyl-L-arabinose and 2,3,4-Tri-*O*-methyl-D-xylose from Component 1.—The sirup (180.0 mg.), $[\alpha]^{23}D$ −8.3° (*c*, 1.8 in water) and −5.5° (*c*, 1.8 in methanol), when examined on a paper chromatogram using methyl ethyl ketone–water azeotrope, showed the presence of two overlapping spots, both having an R_f value close to 0.80. The leading edge of the spot was mauve and the trailing edge light brown, turning pink on standing. Comparison with authentic samples indicated that this was consistent with the behavior of a mixture of 2,3,5-tri-*O*-methyl-L-arabinose and 2,3,4-tri-*O*-methyl-D-xylose.[28] The rotations of these pure compounds are, respectively, −38.5 and +18.5° (*c* 1 in methanol),[29] which shows component 1 to be a mixture of 58% tri-*O*-methyl-D-xylose (104 mg.) and 42% tri-*O*-methyl-L-arabinose (76 mg.).

The sirup (180 mg.) containing the two sugars was dissolved in methanol (5 ml.) containing hydrogen chloride (1%). In 3 hr. at room temperature the observed rotation changed from −0.10 to +0.48° (constant). The acid was neutralized with silver carbonate, and the filtrate and methanol washings were evaporated to a sirup which was added to the top of a hydrocellulose–cellulose (1:1) column and separated into two components as before using methyl ethyl ketone–water azeotrope. After 5 hours, fractions (6 ml.) were collected at 10-minute intervals, and placing samples on paper and spraying with the *p*-anisidine reagent[27] showed brown spots for tubes 10–19 but no spots for tubes 1–9. However, a sample taken from tube 6 gave a strong Molisch test, so the eluates in tubes 2–9 (component 1a) and tubes 13–19 (component 1b) were evaporated separately.

Component 1a: 2,3,5-Tri-*O*-methyl-L-arabinose.—The sirup obtained (65 mg.), having $[\alpha]^{23}D$ +20° (*c* 1 in methanol), was heated for 8 hr. with 0.1 *N* sulfuric acid (5 ml.) on a steam-bath. Neutralization, by passage through Duolite A4 resin, and concentration of the effluent yielded a sirup (42 mg.) $[\alpha]^{22}D$ −35° (*c* 0.8 in methanol). Examination on a paper chromatogram showed that the major component was 2,3,5-tri-*O*-methyl-L-arabinose with a small amount of 2,3,4-tri-*O*-methyl-D-xylose as a contaminant.

The sirup was dissolved in water (2 ml.) and bromine (10 drops) added. The flask was stoppered and oxidation allowed to proceed in the dark for 50 hr. at room temperature; the reaction was completed by warming at 55° for 6 hr. The reaction mixture was diluted with water (10 ml.) aerated to remove excess bromine and neutralized with silver carbonate. Filtration, passage of hydrogen sulfide to remove silver ions, filtration and concentration yielded a sirupy lactone which was distilled, b.p. (bath. temp.) 125–145° (0.02 mm.). The lactone was dissolved in methanol (5 ml.), saturated with ammonia at 0° and kept at 5° for 24 hr. Removal of the solvent yielded 2,3,5-tri-*O*-methyl-L-arabonamide, m.p. and mixed m.p. 132–133°,[12] after recrystallization from acetone–petroleum ether.

Component 1b: 2,3,4-Tri-*O*-methyl-D-xylose.—The sirup (35 mg.), $[\alpha]^{22}D$ +14° (*c* 2.4 in methanol), was chromatographically pure and corresponded with 2,3,4-tri-*O*-methyl-D-xylose. The sirup had a sweet smell suggestive of ketone polymers, which probably accounts for the low rotation. The sirup was therefore purified by sheet paper chromatography using methyl ethyl ketone–water azeotrope as the developing solvent. Location of the component and extraction with acetone yielded 2,3,4-tri-*O*-methyl-D-xylose as a colorless sirup (16 mg.). The anilide was prepared but failed to crystallize satisfactorily. The free sugar was therefore recovered by hydrolysis of the anilide with Amberlite IR 120 in aqueous solution[30] and was oxidized with bromine as before. Distillation of the sirupy product, b.p. (bath. temp.) 75–95° (0.02 mm.), gave 2,3,4-tri-*O*-methyl-D-xylono-δ-lactone which crystallized spontaneously, m.p. 44–48°, and after drying on a porous tile it had m.p. and mixed m.p. 48–50°.[11]

(b) Component 2; 2,3-Di-*O*-methyl-D-xylose.—Evaporation of the eluates containing this component yielded a colorless sirup (768 mg.), $[\alpha]^{23}D$ +2.1° (*c* 0.9 in water),[13] which was shown to be chromatographically pure and to correspond with 2,3-di-*O*-methyl-D-xylose.

The anilide, prepared in the usual way and recrystallized from ethyl acetate–petroleum ether, formed long needles of 2,3-di-*O*-methyl-D-xylose anilide, m.p. and mixed m.p. 141–142°, $[\alpha]^{23}D$ +188° (*c* 1.2 in ethyl acetate).[13]

(c) Component 3: 3-*O*-Methyl-D-xylose.—Placing the samples of the eluates on paper and spraying showed that tubes 87–140 contained sugar but that there appeared to be two overlapping fractions. Samples from tubes 106–120 were placed on a paper chromatogram and developed overnight with methyl ethyl ketone–water azeotrope so that the sugars travelled about two-thirds the length of the paper. The chromatogram indicated that the majority of these tubes contained 2- and 3-*O*-methyl-D-xylose and that pure fractions might be obtained by combining tubes 87–105 and 121–140. (Chromatographically, 3-*O*-methyl-D-xylose travels slightly faster than the 2-methyl compound and gives a pale brown color with the *p*-anisidine reagent, whereas the latter gives a dark reddish-brown spot.)

The sirup (73.7 mg.) obtained by the evaporation of the eluate in tubes 87–105 was shown to be chromatographically pure and to correspond to 3-*O*-methyl-D-xylose. Nucleation of the sirup caused crystallization, and pure 3-*O*-methyl-D-xylose was obtained by recrystallization from acetone, m.p. and mixed m.p. 95–96°, $[\alpha]^{23}D$ +25.8° (*c* 1.5 in methanol, equilibrium).[10]

(d) Component 4; 2-*O*-Methyl-D-xylose.—The sirup (57.0 mg.) obtained by evaporation of the eluate in tubes 121–140 crystallized spontaneously. Recrystallization from ethyl acetate yielded 2-*O*-methyl-D-xylose, m.p. and mixed m.p. 132–133°, $[\alpha]^{23}D$ −21° (*c* 1.1 in methanol, initial value) changing to +34.3° (equilibrium value).

Evaporation of the eluate in tubes 106–120 furnished a sirupy mixture of 2-*O*- and 3-*O*-methyl-D-xylose (82.0 mg.), $[\alpha]^{23}D$ +24.4° (*c* 1.6 in methanol). Hence, on the basis of optical rotation, the mixture contained 37% 2-*O*-methyl-D-xylose (30.4 mg.) and 63% 3-*O*-methyl-D-xylose (51.6 mg.). This brings the total weight of the 2-*O*-methyl isomer to 87.4 mg. and of the 3-*O*-methyl isomer to 125.3 mg.

(e) Component 5; (?) D-Xylose.—Concentration of the contents of tubes 176–250 yielded a small amount of brown sirup (17.1 mg.) which did not crystallize. This was shown chromatographically to contain only xylose. The rotation was $[\alpha]^{23}D$ +14° (*c* 0.4 in methanol) and on this basis the weight of xylose amounted to 12.5 mg. This amount of xylose may not be of any constitutional significance.

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