chromatographed on silicic acid. The traces of methyl Cellosolve left in the residual material were eluted in the first benzene fraction. The results are reported in Table I.

In a general way, the starting materials were eluted by mixtures of benzene and ether 49:1, 19:1 or 9:1. Methyl 2,3-anhydro-4,6-*O*-benzylidene-α-D-allopyranoside and methyl 2,3-anhydro-4,6-*O*-benzylidene-α-D-mannopyranoside were eluted immediately afterward with the same mixtures of solvents. Methyl 4,6-*O*-benzylidene-2-*O*-*p*-tolylsulfonyl-α-D-glucopyranoside was eluted by mixtures of benzene and ether 19:1 and 9:1, whereas the 2-*O*-methylsulfonyl derivative required the same solvents in proportions 4:1 and 2:1.

Methyl 4,6-*O*-benzylidene-3-*O*-methylsulfonyl-α-D-glucopyranoside was eluted with the same mixture of solvents in 1:1 proportion and was recrystallized from a mixture of acetone, ether and pentane as prismatic needles, m.p. 145–146°, $[\alpha]^{25}D$ +90 ± 1° (in chloroform, *c* 2.73).[23] *Anal.* Calcd. for $C_{15}H_{20}O_8S$: C, 49.89; H, 5.59; S, 8.90. Found: C, 49.91; H, 5.67; S, 8.87. In admixture with authentic material, the m.p. was not depressed. The compound was characterized by acetylating 50 mg. with acetic anhydride in pyridine solution in the usual manner. After crystallization from a mixture of ether and pentane, 55 mg. (98%) of methyl 2-*O*-acetyl-4,6-*O*-benzylidene-3-*O*-methylsulfonyl-α-D-glucopyranoside, m.p. 153–154°, was obtained, $[\alpha]^{25}D$ +72 ± 2° (in chloroform, *c* 1.08), showing no depression of the m.p. in admixture with authentic material.[7]

Methyl 4,6-*O*-benzylidene-α-D-altropyranoside was eluted with pure ether. Recrystallization from a mixture of acetone, ether and pentane gave shiny platelets, m.p. 173–174°, $[\alpha]^{24}D$ +114 ± 2° (in chloroform, *c* 1.02).[24] *Anal.* Calcd. for $C_{14}H_{18}O_6$: C, 59.57; H, 6.43. Found: C, 59.46; H, 6.54. In admixture with authentic material the m.p. was not depressed.

Methyl 6-*O*-benzoyl-2-*O*-methylsulfonyl-α-D-glucopyranoside was eluted with a mixture of ether and ethyl acetate 4:1, whereas methyl 4,6-*O*-benzylidene-α-D-glucopyranoside was eluted with a mixture of ether and methanol 9:1.

In view of the low yields in crystalline material resulting from the reaction of methyl 3,4,6-tri-*O*-benzoyl-3-*O*-methylsulfonyl-α-D-glucopyranoside and methyl 2,4,6-tri-*O*-benzoyl-2-*O*-methylsulfonyl-α-D-glucopyranoside due to probable loss of debenzoylated product during the extraction procedure, the mixture resulting from the reaction was evaporated to dryness. The residue was refluxed with an excess of 2 *N* HCl for two hours. The solution then was passed through a column of Dowex 50 in acid form and a column of Amberlite IRA 400 in carbonate form and evaporated *in vacuo*. The residue was chromatographed on Whatman No. 54 and No. 1 papers and developed with a mixture of butan-2-one, water and concentrated ammonia in proportions 55:4:1.5. The standards used were glucose, mannose, a mixture of altrose and altrosan, and allose. Identification of the spots was made with alkaline silver nitrate. No spot corresponding to mannose could be observed with the first compound.

For the second compound no very satisfactory solvent was found for the separation of D-allose from D-altrose. However, it was possible to ascertain that no significant amount of D-allose had been formed from D-glucose during the course of the reaction.

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BOSTON, MASS.

(23) Honeyman and Morgan[16] reported m.p. 142–143°, $[\alpha]^{17}D$ +90° (in chloroform, *c* 1.0).

(24) Robertson and Griffith[12] reported m.p. 169–170° and $[\alpha]D$ +126.8° (in chloroform, *c* 0.567); Richtmyer and Hudson[13] reported m.p. 169–170°, $[\alpha]D$ +115.0° (in chloroform, *c* 2.0).

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF BRITISH COLUMBIA]

The Constitution of the Hemicellulose of Sitka Spruce (*Picea sitchensis*). II. Structure of the Mannan Portion[1]

BY G. G. S. DUTTON AND K. HUNT[2]

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Extraction of sitka spruce chlorite holocellulose with aqueous sodium hydroxide yielded a hemicellulose fraction rich in D mannose and D-glucose. Methylation studies show that the sugar units are joined by β-1,4-links and that the chains, which are apparently unbranched, contain 40–60 sugar residues. One sample contained D-galactose in a terminal position. The structural features of these glucomannans are discussed.

It is commonly stated that one of the main chemical differences between the hemicelluloses of soft and hard woods is that the latter are predominantly xylans while the former are mixtures of xylans and appreciable amounts of mannans.[3] It is apparent that these two terms are oversimplifications in that there has been no evidence for the existence of a pure mannan in a wood hemicellulose and in the case of western hemlock hemicellulose the xylan fraction has been shown to be an arabomethoxyglucuronoxylan.[4] The observation[5] that the mannan-rich fraction of hemicelluloses is more soluble in sodium hydroxide than in potassium hydroxide has enabled a good separation of these components to be made. In the present work, sitka spruce chlorite holocellulose was extracted with 10% potassium hydroxide and the washed and air-dry residue re-extracted with 18% sodium hydroxide. The composition of the potassium hydroxide extract has been discussed and the structure of the aldobiouronic acid proved.[6] This paper is concerned with the nature of the sodium hydroxide extract and the approach to the problem has been in two ways. In one instance the crude extract was methylated with dimethyl sulfate and sodium hydroxide[7] and since the partially methylated material did not readily separate, the solution was dialyzed and evaporated between each of six successive treatments. Three further treatments with Purdie reagents[8] yielded the fully methylated polysaccharide which was fractionated from chloro-

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(2) We acknowledge with thanks a grant from the National Research Council of Canada and the award of a summer research associateship.

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form solution by the addition of petroleum ether. The material appeared homogeneous and had $[\alpha]^{22}D$ −20° (*c* 2 in chloroform). This is designated polysaccharide I. Following methanolysis of the methylated polysaccharide the methyl glycosides were hydrolyzed with sulfuric acid (1 *N*) for 55 hr. and the methylated sugars separated on a cellulose–hydrocellulose[9] column after deionization with Duolite A-4 resin.[10] It was apparent that the first fraction contained much unhydrolyzed material and this was rehydrolyzed with sulfuric acid (1 *N*) for 96 hr. Other workers also have found difficulty in hydrolyzing mannose-containing polysaccharides,[11] but a recent publication has shown that hydrolysis proceeds readily in formic acid.[12] When the rehydrolyzed material was separated on a cellulose column it was apparent that the hydrolyzed methylated polysaccharide yielded three main components. These were identified as 2,3,4,6-tetra-*O*-methyl-D-mannose, principally by optical rotation[13]; 2,3,6-tri-*O*-methyl-D-glucose obtained as the crystalline sugar[14]; and 2,3,6-tri-*O*-methyl-D-mannose, characterized as its di-*p*-nitrobenzoate.[11] There was also a small fraction having an R_t value corresponding to 2,3-di-*O*-methyl-D-mannose. Contrary to previous claims[11] it was found quite practical to separate the tri-*O*-methylglucose from the tri-*O*-methylmannose by column chromatography, although it is not claimed that the separation was quantitative. It also was found that a paper chromatogram developed in butanone–water azeotrope clearly showed the tri-*O*-methylhexose fraction to consist of at least two components.[11]

From this experimental evidence it may be deduced that one of the components of sitka spruce hemicellulose is a glucomannan which is essentially linear, and consists of D-glucose and D-mannose residues joined by β-1,4 linkages and in which the chains are terminated by mannose residues. The small amount of dimethylmannose may be of structural significance and indicate a branch point or it may result from demethylation[15] or incomplete methylation.[16] The quantitative data suggest that the chains contain approximately 40 sugar units and that about 1 in 15 is a glucose unit. This is in fair agreement with an analysis of the original sodium hydroxide extract which showed 90% mannose and 10% glucose when a hydrolyzed sample was analyzed by the phenol–sulfuric acid method.[17]

The work described above was essentially of a preliminary nature and while this was carried out the second method was investigated. This consisted of attempting a preliminary fractionation of the sodium hydroxide extract by precipitation of the acetate. It was found impossible to acetylate the polysaccharide by the customary formamide procedure,[18] but the material slowly was acetylated by mixing the alcohol-wet polysaccharide with pyridine and acetic anhydride and stirring on the steam-bath.[5] A certain amount of polysaccharide remained unreacted and this was filtered off and added to a subsequent batch. By the fractionations shown in Table III there was obtained a fraction designated $2b_2$ which appeared homogeneous and had $[\alpha]D$ −30°. This acetate was methylated by Haworth's procedure, but since the partially methylated polysaccharide separated as a solid even after the first methylation no dialysis was necessary. After six methylations the product was methylated thrice with Purdie reagents and fractionated from chloroform to yield an apparently homogeneous material having $[\alpha]^{22}D$ −22.5°. This is designated polysaccharide II. This methylated polysaccharide was hydrolyzed with sulfuric acid for 53 hr. and again yielded an incompletely hydrolyzed portion which was retreated with formic acid.[12] Separation of the sugars on the column showed the mixture to contain 2,3,4,6-tetra-*O*-methyl-D-mannose (trace); 2,3,4,6-tetra-*O*-methyl-D-galactose, characterized as crystalline N-phenyl galactosylamine[19]; 2,3,6-tri-*O*-methyl-D-glucose, identified as the crystalline sugar[14]; 2,3,6-tri-*O*-methyl-D-mannose, identified as its di-*p*-nitrobenzoate[11]; and a di-*O*-methylhexose corresponding chromatographically with 2,3-di-*O*-methyl-D-mannose. Although these results do not permit the deduction of a unique structure, they indicate that the D-glucose and D-mannose units are joined through β-1,4-linkages and that the D-galactopyranose units must occupy terminal positions. The quantitative data indicate that the chains contain approximately 55 hexose residues. Although there have been many instances of D-galactose being found in hemicellulose hydrolysates[3] the only other instances of a methylated-D-galactose unit being obtained from a methylated hemicellulose preparation are in the case of methylated western hemlock polysaccharide B which has been found to contain 2,3,4,6-tetra-*O*-methyl-D-galactose and 3,4,6-tri-*O*-methyl-D-galactose[20] and methylated loblolly pine hemicellulose which yields 2,3,4,6-tetra-*O*-methyl-D-galactose.[21] It is possible that in the case of sitka spruce hemicelluloses the D-galactose units are part of a relatively short chain polysaccharide which may be lost readily during the dialysis operations involved in the methylation procedure for polysaccharide I. It should be emphasized that certain types of regenerated cellulose tubing which are commercially available have been made under conditions designed to give maximum permeability. This means that during dialysis one may unwittingly be fractionating short chain mate-

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rial from long chain material. If the material is chemically homogeneous this only results in a lower yield, but where the material is not chemically homogeneous it may lead to erroneous interpretations. In the present work it is considered significant that the preparation of polysaccharide II involved no dialysis. It is also known[5] that in pulping operations the arabinose and galactose units are the most readily eliminated, demonstrating the weakness of the galactoside linkage or the solubility of the galactose-containing polysaccharide. As a further example it may be noted that recent preparations in this Laboratory of L-arabinose by partial hydrolysis of mesquite gum[22] have given good results when dialysis was carried out using Dennison sheet cellophane type 3100, but when commercial dialysis tubing from several sources was employed the arabinose solution was contaminated with large amounts of oligosaccharides.

The constitution of the two polysaccharides described here shows that they have many features in common and also that they resemble cellulose and typical xylans in having β-1,4-linkages. It is doubtless this close structural similarity which makes efficient separation of these polysaccharides so difficult. The structure of glucomannans from several plant sources have been determined (see ref. 12), but relatively little is known about those from wood hemicelluloses. There have been preliminary reports on the glucomannan fractions of loblolly pine,[21] western hemlock wood,[20] western hemlock sulfite pulp[23] and, as the present work was being completed, a study of sitka spruce glucomannans was published.[12] These results[12] conform to the same pattern as those discussed in the present paper except that no galactose was detected in the methylated glucomannan and there was also present a β-1,4-linked glucan which, however, may have arisen from partial degradation of the cellulose. From the evidence presently at hand, it appears that glucomannans from wood and other plant sources consist of a closely related group of polysaccharides which differ mainly in the ratio of glucose to mannose. The constitutional significance of the galactose units will require further study.

Experimental

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

Extraction of Glucomannan.—Sitka spruce holocellulose was extracted with potassium hydroxide (10%) and the residue washed with water.[6] This air-dried material was then extracted with sodium hydroxide (18%, 1.5 and 0.5 l.) for 4 hr. and the filtrate acidified with acetic acid. The gelatinous precipitate was centrifuged after standing overnight, washed with water and dried by solvent exchange. The yields from 100 g. of holocellulose varied between 4 and 7 g. and had $[\alpha]_D$ −22 to −28° (*c* 1 in 6% NaOH). When a sample was hydrolyzed and examined chromatographically, sugars corresponding to glucose and mannose were observed. When a sample was analyzed quantitatively by the phenol–sulfuric acid method[17] the ratio of mannose to glucose was found to be 9:1.

Methylation of Glucomannan.—The glucomannan (7 g.) was suspended in sodium hydroxide (18%, 100 ml.). On warming and shaking for 4 hr. all of the polysaccharide had dissolved to give a thick brown solution. Solid sodium hydroxide (12 g.) was added and then dimethyl sulfate (80 ml.) and sodium hydroxide (30%, 200 ml.) were added with vigorous stirring over 3 hr. After one-third of the dimethyl sulfate had been added, the solution became extremely viscous and acetone was added. Finally, hot water (150 ml.) was added and the flask heated on a boiling water-bath to remove the acetone. The partially methylated material tended to settle out on the surface as brown flocs, but since it could not be quantitatively removed the solution was dialyzed against running tap water overnight. The contents of the dialysis bag was concentrated and remethylated. This process was repeated six times and the final sirup dissolved in chloroform. Evaporation of the solvent left a brown resin which was dissolved in acetone (50 ml.) and methyl iodide (50 ml.). It was necessary to allow the resin to stand overnight with the acetone to obtain a clear solution. Drierite (2 g.) was added and silver oxide (20 g.) was added in small portions over 4.5 hr. to the refluxing and stirred solution. The polysaccharide was recovered by repeated extraction with acetone and the methylation with Purdie reagents repeated twice more.

Fractionation of the Methylated Polysaccharide.—The crude methylated material was dissolved in dry chloroform (100 ml.) and dry diethyl ether (100 ml.) added. (It was observed that when the polysaccharide was dissolved in warm chloroform (50 ml.), the solution set to a gel on standing overnight.) Fractionation was achieved by the addition of dry petroleum ether (30–60°) to the mechanically stirred solution and the results are shown in Table I. From these results it was concluded that the material was essentially homogeneous having $[\alpha]^{22}D$ −20° (*c* 1 in chloroform).

TABLE I

FRACTIONAL PRECIPITATION OF METHYLATED SITKA SPRUCE GLUCOMANNAN

Fraction	Total petroleum ether added, ml.	Weight, g.	$[\alpha]^{22}D$(CHCl₃) (*c* 2)	OMe, %
1	150	0.11	..[a]	..
2	200	0.68	−19°	43.8
3	250	2.44	−21	44.6
4	350	0.13	−15	..

[a] This fraction contained inorganic matter.

Methanolysis of Glucomannan.—Fractions 3 (2.4 g.), polysaccharide I, was dissolved in methanol (100 ml.) containing hydrogen chloride (2.5%) and refluxed. The optical rotation changed from −1.02° to +1.90° (1 dm.) corresponding to a final rotation of $[\alpha]^{22}D$ +79°. After neutralization (Ag_2CO_3) the methanol was evaporated. The sirup was dissolved in sulfuric acid (1 *N*, 72 ml.) and heated on the steam-bath under reflux. The optical rotation changed from $[\alpha]^{22}D$ +40° to +25° (constant, 55 hr.) and acid was removed by passage through a column of Duolite A-4 resin. Evaporation of the neutral eluate yielded a sirup (2.1 g.) containing a mixture of methylated sugars.

Separation of the Methylated Sugars.—The sirup (2.1 g.) was added to a cellulose–hydrocellulose column (40 cm. by 2.8 cm. i.d.), two drops of Sudan IV dye was added to mark the solvent front, and the column developed with butanone–water azeotrope. The column was jacketed at 30°, the front time was 3.5 hr. and the rate of flow 25 ml./hr. Tubes 1 (from the dye)–35 were collected at 10-min. intervals and every 30 min. thereafter with the results shown in Table II. It was apparent that the hydrolysis was incomplete, but from paper chromatography it was shown that fraction C was essentially pure 2,3,6-tri-*O*-methyl-D-man-

TABLE II

INITIAL SEPARATION OF METHYLATED SUGARS

Tube number	Fraction	Wt., mg.	$[\alpha]^{22}D$ in MeOH
1–10	A	430	67°
11–30	B	820	41.5
31–50	C	530	12
60–70	D	23	16
		1803	
	Recovery	86%	

(22) E. V. White, THIS JOURNAL, **69**, 715 (1947).

(23) J. K. Hamilton and H. W. Kircher, 132nd A.C.S. Meeting, New York, N. Y., Sept., 1956.

nose. Fraction A was re-hydrolyzed with sulfuric acid (1 *N*) on the steam-bath for 96 hr. and, after neutralization, re-chromatographed on the column when it was shown to yield a further quantity of tri-*O*-methyl sugars and a small amount of a tetra-*O*-methyl compound. Fraction B was shown by paper chromatography (butanone–water) to consist of two components and this fraction was also separately re-chromatographed, but without further hydrolysis. This material was collected in tubes 16–40 (10-min. intervals) and after standing several weeks, the contents of tubes 17–20 spontaneously crystallized.

Identification of the Components.—(a) **Component 1. 2,3,4,6-Tetra-*O*-methyl-D-mannose** was obtained as a sirup (42 mg.), having $[\alpha]^{22}D$ +25.2° (*c* 0.8 in methanol) and a slight positive rotation in water. A synthetic sample of 2,3,4,6-tetra-*O*-methyl-D-mannose which was a sirup but chromatographically pure had $[\alpha]^{22}D$ +25.9° (*c* 3.5 in methanol), in good agreement with the quoted value of $[\alpha]D$ +27.3° for crystalline material.[13] A sample of component 1 when chromatographed on Whatman No. 1 paper and in butanone–water azeotrope traveled 30.8 cm. and 27.9 cm. measured from the origin to the leading and trailing edges of the spot. These values were the same for an authentic sample of 2,3,4,6-tetra-*O*-methyl-D-mannose, while 2,3,4,6-tetra-*O*-methyl-D-glucose, run on the same chromatogram, gave values of 31.6 and 28.3 cm. The solvent front traveled 38.0 cm.

(b) **Component 2. 2,3,6-Tri-*O*-methyl-D-glucose.**—A total of 103 mg. of crystalline material was obtained having m.p. and mixed m.p. 119–120° on recrystallization from ether and $[\alpha]^{22}D$ 67.5° (*c* 1.5 in water, equil.).[14]

(c) **Component 3. 2,3,6-Tri-*O*-methyl-D-mannose** was identified by using the material in tubes 21–30 obtained by passing fraction B through the column a second time. This material was chromatographically identical with that of fraction C and the major portion of the rehydrolyzed fraction A. It was identified by the preparation of the di-*p*-nitrobenzoate which could be recrystallized from a large volume of methanol in stout needles or from a small volume of ethyl acetate in fine hair-like needles. In either case the m.p. and mixed m.p. was 188–189°.[11] The total amount of 2,3,6-tri-*O*-methyl-D-mannose obtained was 1452 mg.

(d) **Component 4**, *i.e.*, Fraction D (Table II) (23 mg.) had an R_f value similar to 2,3-di-*O*-methyl-D-mannose but was not otherwise examined.

Acetylation of Glucomannan.—Acetylation by the method of Carson and Maclay[18] failed to yield any acetate because of the insolubility of the polysaccharide in formamide. Accordingly Hamilton's method[5] was used with better success. Alcohol-wet hemicellulose (2 g.) was mixed with pyridine (55 ml.) and acetic anhydride (33 ml.). The mixture was stirred and heated on a steam-bath in an inert atmosphere for 20 hr. The dark brown solution was filtered from undissolved hemicellulose and poured into water (1 l.) containing a little hydrochloric acid. The precipitated acetate was centrifuged, washed and dried by solvent exchange. The unreacted hemicellulose was added to the next portion acetylated until a total of 10 g. of hemicellulose had been so treated. The weight of acetate was 6.0 g. and there remained 2.9 g. unreacted.

Fractionation of Acetylated Glucomannan.—Acetylated polysaccharide (6.0 g.) was dissolved in chloroform (250 ml.) and fractionally precipitated by petroleum ether (30–60°). Fractions 1 and 2 were redissolved in chloroform and reprecipitated to yield sub-fractions 1a, 1b, etc. Since fraction 2b was large, this was reprecipitated again yielding $2b_1$ etc. These results are shown in Table III.

Methylation of Acetylated Glucomannan.—The acetylated polysaccharide, fraction $2b_2$ (2.6 g.), was dissolved in acetone (60 ml.) and the solution warmed to 40–45°. To this solution there were added with vigorous mechanical stirring sodium hydroxide (30%, 10 ml.) and dimethyl sulfate (3.5 ml.) every 15 min. until a total of 8 additions had been made. The solution was stirred for 1 hr. longer and then 8 further additions were made together with acetone (*ca.* 50 ml.) to replace that lost by evaporation. After the final addition the mixture was stirred for 1 hr. and then the water-bath raised to boiling point and the stirring stopped. When all the acetone had been removed, boiling water (*ca.* 200 ml.) was added to dissolve the salts and the partly methylated polysaccharide was obtained in small lumps. The aqueous layer was decanted through glass wool leaving the majority of the polysaccharide in the flask and that on the filter was dissolved in acetone and returned to the flask. The methylation was repeated with four further batches of sodium hydroxide and dimethyl sulfate, but the polysaccharide was isolated only after the fourth and sixth additions as described above. The product from the sixth methylation was freely soluble in chloroform and acetone. It was dissolved in chloroform (200 ml.) and dried by distillation of the solvent; yield of brown resin (*ca.* 2 g.). This was dissolved in acetone (20 ml.) and methyl iodide (20 ml.) and Drierite added (2 g.). Methylation was continued for 10 hr. with the periodic addition of silver oxide (8 g.) and the isolated product was freely soluble in methyl iodide. Methylation was repeated without the addition of acetone and there was finally obtained a brown resin (1.75 g.). Fractionation with petroleum ether (30–60°) from an ether–chloroform (1:1) solution showed the material (polysaccharide II) was apparently homogeneous and had $[\alpha]^{22}D$ −22.5° (*c* 4 in chloroform), OMe 42.2%.

TABLE III

FRACTIONATION OF ACETYLATED GLUCOMANNAN

Fraction	Total petroleum ether added, ml.	Weight, g.	$[\alpha]^{22}D$ ($CHCl_3$)
1	125	0.85[p]	..
2	225	4.35[q]	..
3	425	0.14	−29.4°
1a	125	0.54	[r]
1b	625	0.11	−34.9
2a	200	0.42	−41.8
2b	350	3.42[s]	..
2c	900	0.41	−29.7
$2b_1$	250	0.74	−23.2
$2b_2$	400	2.68	−30.0
$2b_3$	1000	Trace	..

[p] Redissolved in 175 ml. of chloroform. [q] Redissolved in 400 ml. of chloroform. [r] Cloudy, rotation not observable. [s] Redissolved in 200 ml. of chloroform.

Hydrolysis of Methylated Glucomannan.—The methylated product obtained above (0.9 g.) was dissolved in sulfuric acid (1 *N*, 20 ml.) and heated under reflux on the steam-bath for 53 hr. It was not possible to follow the optical rotation, but the final solution was dextrorotatory. The acid was neutralized by passage through Duolite A-4 resin and the neutral eluate evaporated to give a mixture of methylated sugars.

Separation of Neutral Sugars.—The sirup was added to a cellulose–hydrocellulose column as before and developed with butanone–water azeotrope. The first 35 tubes were collected at 15-min. intervals and the remainder at 30 min. Paper chromatography indicated that the following tubes should be combined: component 1, tubes 10–15 (144 mg.), $[\alpha]D$ +53.5° (*c* 2.8 in methanol); component 2, tubes 16–19 (70 mg.), $[\alpha]D$ +65.5° (*c* 1.4 in ethanol); components 3 and 4, tubes 20–40 (890 mg.), $[\alpha]D$ +16.5° (*c* 3 in methanol); component 5, tubes 52–65 (31 mg.), $[\alpha]D$ +12° (*c* 1 in methanol). It was apparent that tubes 10–15 contained some unhydrolyzed material and this was accordingly rehydrolyzed with formic acid (98%).[12] The recovered sirup (128 mg.) was separated on the column and a tetramethyl sugar (17 mg.) obtained in tubes 7–13 (10-min. fractions). The remainder of the material was added to the corresponding fractions from the first separation.

Identification of the Components. (a) **Component 1. 2,3,4,6-Tetra-*O*-methyl-D-mannose** (17 mg.), was tentatively identified by its behavior on a paper chromatogram, and the fact that it gave a positive optical rotation in methanol.[13]

Component 2. 2,3,4,6-tetra-*O*-methyl-D-galactose (70 mg.), was characterized by its optical rotation $[\alpha]D$ +65.5° (*c* 1.4 in ethanol) and by the preparation of the crystalline N-phenyl galactosylamine, m.p. and mixed m.p. 192–194°.[19]

Components 3 and 4. 2,3,6-Tri-*O*-methyl-D-glucose and -D-mannose.—A portion of tubes 20–40 was treated with 1% methanolic hydrogen chloride at room temperature[11] when the optical rotation changed from $[\alpha]D$ +11.1° to −3.4° in 13 hr. The mixture of methyl 2,3,6-tri-*O*-methyl-D-glucofuranoside and 2,3,6-tri-*O*-methyl-D-mannose was separated by column chromatography and the mannose de-

rivative identified as the di-*p*-nitrobenzoate, m.p. and mixed m.p. 187–189°.[11] The furanoside was hydrolyzed, deionized with Duolite A-4 and evaporated to yield a sirup which crystallized on seeding. The 2,3,6-tri-*O*-methyl-D-glucose had m.p. and mixed m.p. 117–120°.[14] From the initial and final rotations of the furanoside mixture it may be calculated that the glucose component amounts to 10-20% of the trimethyl sugars.

Component 5 was tentatively identified by chromatography as 2,3-di-*O*-methyl-D-mannose.

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[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, PURDUE UNIVERSITY]

Oxidation of Alginic Acid with Hypochlorite at Different Hydrogen Ion Concentrations[1]

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Alginic acid is oxidized more slowly by hypochlorite at *p*H levels of 3, 5, 7, 9 and 11 than is corn starch amylopectin. However, in correspondence with the amylopectin, oxidation proceeds most swiftly at *p*H 7 and extensive attack occurs at carbon atoms C_2 and C_3 as evidenced by the isolation of oxalic, L-tartaric and D,L-tartaric acids from the oxidized and hydrolyzed products.

Commercial polysaccharides frequently are subjected to the action of oxidizing agents either for the purpose of bleaching or for modification of properties. In previous publications[2] this Laboratory has described the action of hypochlorite on starch and on methyl 4-*O*-methyl-α,D-glucopyranoside and methyl 2-*O*-methyl-α-D-glucopyranoside.

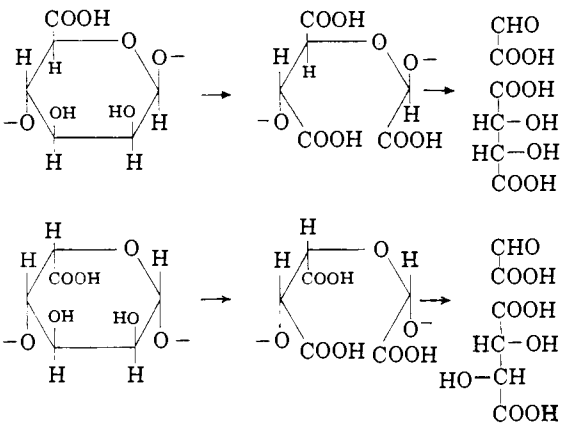
To obtain information on how changes in the grouping at carbon atom C_6 can affect the oxidation, attention is now directed at alginic acid. This polysaccharide is produced commercially in the United States from the seaweed *Macrocystis pyrifera*. It is a linear glycuronoglycan which contains[3] roughly 70% D-mannuronic acid units, presumably linked β(1 → 4), and about 30% L-guluronic acid units in, as yet unestablished but presumed, 1→4-linkages. The order of occurrence of the two types of uronic acid units is not known. However, because each sugar unit contains a carboxyl group at the C_6-position it is of interest to determine how this grouping affects the attack on the sugar units by hypochlorite.

On treatment with hypochlorite at different *p*H levels the polysaccharide is oxidized as shown in Fig. 1. It is observed, at once, that at all *p*H levels hypochlorite oxidation of alginic acid proceeds in a manner similar to the hypochlorite oxidation of amylopectin. Significantly, oxidation proceeds most swiftly at *p*H 7. However, major differences are also apparent. First, it is noted that at all *p*H levels oxidation proceeds more slowly than with corn amylopectin. Furthermore, the amount of carbon dioxide produced during the oxidation (Table I) is higher for alginic acid at all *p*H levels than for amylopectin.

TABLE I

*p*H	3	5	7	9
Moles of carbon dioxide per mole uronic acid unit per 3 moles of hypochlorite consumed	1.39	1.16	0.72	1.27

Correspondingly, the oxidative attack to cleave carbon atoms C_2 and C_3 should be less if it proceeds according to the same mechanism as postulated for amylopectin. If oxidative attack occurs in this fashion the products should be glyoxylic and L-tartaric acids from the L-guluronic acid units and glyoxylic and *meso*-tartaric acids from the D-mannuronic acid units.



Evidence for these products is obtained by isolations from the hydrolyzate of hypochlorite-oxidized algin. Presence of glyoxylic acid is indicated by isolation of oxalic acid after further oxidation of the hydrolysis products with hypoiodite (Table II). L-Tartaric acid can be isolated directly from the hydrolyzate, but *meso*-tartaric acid undergoes rearrangement[4–6] under the conditions of hydrolysis and is converted to the D,L-tartaric acid, which is isolated (Table II).

TABLE II

*p*H	Mole tartaric acid per mole uronic acid unit	Mole oxalic acid per mole uronic acid unit
3	0.032	0.035
5	.034	.047
7	.054	.068
9	.060	.081

The yield of oxalic and tartaric acids suggests that only about 6–7% of the hypochlorite not

(1) Journal Paper No. 1293 of the Purdue Agricultural Experiment Station, Purdue University, Lafayette, Ind.

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