ponent is a concentration anomaly.[37–39] This anomaly can be explained if one assumes that the sedimentation coefficient of the slower component is greatly dependent on the concentration as observed above for that of the faster moving component.

For simplicity let us assume that the equation obtained by Johnston and Ogston[40] is valid[37]: $C_s^{obs}/C_s^0 = (S_f - S_{s,mixt})/(S_f - S_s)$ where C_s^{obs} and C_s^0 are the observed and initial concentrations of the slow component; $S_{s,mixt}$ and S_s are the sedimentation coefficients of the slow component in the presence of the fast component and alone; and S_f is the sedimentation coefficient of the fast component. Again for simplicity, let us assume that in the 0.5% solution the value of $S_{s,mixt}$ is equal to that found in the 1% solution, that is, 17 Svedbergs, and that in the 0.2% solution the value of $S_{s,mixt}$ is equal to that found in the 0.5% solution, that is, 20 Svedbergs. Using the values for the fast and slow components of waxy I amylopectin listed in Table V, the value of C_s^{obs}/C_s^0 for the 0.5% solution is $(141 - 17)/(141 - 20) = 1.03$ and for the 0.2% solution is $(296 - 20)/(296 - 94) = 1.37$. Thus the observed relative concentrations for the slower component in the 0.2% solution may be as much as 4/3 times as large as in the 0.5% solution.

Conclusions

1. Starch granules can be completely dispersed by refluxing in neutral 6 or 8 *M* LiBr solutions without degrading the amylopectin to any detectable extent. 2. In dispersing starch granules by refluxing in amyl alcohol solutions, a buffer must be used. An amylopectin obtained from starch granules dispersed in a buffered, amyl alcohol-saturated solution for 20 hours was less hydrolyzed than an amylopectin obtained from starch granules dispersed for three hours in an unbuffered solution. 3. The above results illustrate that (a) similar light-scattering molecular weights are obtained using *N* KOH, water and 6 *M* LiBr as solvents; (b) sedimentation constants of immature corn amylopectins are extremely large; (c) immature waxy corn amylopectin consistently behaves as a statistically branched polymer (acid hydrolysis studies); and (d) variations in the method of dispersing starch granules with lithium bromide do not appear to alter the molecular weight of the amylopectin. These results indicate that intermolecular hydrogen bonding is absent in the dispersed amylopectin samples and that amylopectins have broad size distributions such as found in A–R–B$_2$ type polymers. 4. Immature waxy corn starch has two components. The fast moving component may be a chemically aggregated amylopectin while the slower moving component may be an amylopectin or glycogen which does not exist as a chemically aggregated "tetramer" or which is an impurity from the pericarp or ovary. The apparent increase in the relative concentration of the slower component when the starch solution is diluted is most likely due to a concentration anomaly in the schlieren ultracentrifuge pattern.

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

The Constitution of the Hemicelluloses of Sitka Spruce (*Picea sitchensis*). I. Composition of the Hemicellulose and Identification of 2-*O*-(4-*O*-Methyl-D-glucopyranosiduronic Acid)-D-xylose[1]

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The hemicelluloses of Sitka spruce which were extracted from the chlorite holocellulose with 10% aqueous potassium hydroxide gave upon hydrolysis D-xylose, D-mannose, D-galactose, D-glucose, (?)L-arabinose and an aldobiouronic acid. All of the neutral sugars except arabinose were identified as crystalline derivatives. The aldobiouronic acid has been identified as 2-*O*-(4-*O*-methyl-α-D-glucuronosyl)-D-xylose.

Sitka spruce sawdust was delignified by treatment with sodium chlorite and the hemicellulose was obtained from the holocellulose by alkaline extraction. Upon hydrolysis the hemicellulose gave an aldobiouronic acid and a mixture of neutral sugars. The acidic component was separated by means of ion-exchange resins and the neutral sugars separated on a cellulose column using 1-butanol saturated with water at 5°. All of the sugars with the exception of arabinose were characterized as crystalline compounds. The aldobiouronic acid

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was shown to be 2-*O*-(4-*O*-methyl-D-glucopyranosiduronic acid)-D-xylose by the following evidence.

Paper chromatographic investigation of the acidic component showed it to contain mainly an aldobiouronic acid, some free acid and small amounts of higher oligosaccharides. A pure sample of the aldobiouronic acid was obtained by separating the acidic mixture on a cellulose column using ethyl acetate–acetic acid–formic acid–water. Cleavage of the aldobiouronic acid with 8% methanolic hydrogen chloride followed by treatment with ammonia yielded the crystalline amide of methyl 4-*O*-methyl-α-D-glucopyranosiduronic acid.[2] After removal of this uronic acid derivative, hydrolysis of the neutral sugar glycoside gave D-xylose characterized as the di-*O*-benzylidene dimethyl acetal.[3]

The point of attachment of the 4-*O*-methyl-D-glucuronic acid unit to the D-xylose was determined from a study of the completely methylated disaccharide, obtained by lithium aluminum hydride reduction[4,5] and methylation of the aldobiouronic ester. Hydrolysis of the methylated disaccharide and chromatographic separation of the components on a cellulose–hydrocellulose column[6] using butanone–water as the irrigating solvent[7] yielded 2,3,4,6-tetra-*O*-methyl-D-glucose and 3,4-di-*O*-methyl-D-xylose. The former was identified as the crystalline sugar[8] and the latter as the crystalline 3,4-di-*O*-methyl-D-xylono-δ-lactone.[9]

This evidence shows that the aldobiouronic acid is 2-*O*-(4-*O*-methyl-D-glucopyranosiduronic acid)-D-xylose and the work of Bishop[10] suggests that the linkage is of the α-type. This acid is identical with that obtained by hydrolysis of other hemicellulose materials and it is observed[11] that 4-*O*-methyl-D-glucuronic acid is commonly, but not always,[12] linked to position 2 of the D-xylose fragment. The existence of other aldobiouronic or higher acids is possible since paper chromatographic examination of the acidic fraction showed several components. The present paper reports the constitution of the main constituent of this mixture.

It is of interest to note that in the case of beech wood, the hemicelluloses may be extracted from the whole wood by 1 *N* aqueous sodium hydroxide,[13] but when this was tried on Sitka spruce only a negligible amount of polysaccharide was isolated. It may well be that this is another illustration of the differences between the hemicelluloses of deciduous and evergreen trees.

Experimental

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

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Isolation of Sitka Spruce Hemicellulose.—The sawdust, as received, was delignified in 100-g. batches by treatment with sodium chlorite and acetic acid.[14] The average yield of holocellulose was 67 g. The original sawdust and the holocellulose when air-dried were found to contain 6.6 and 4.4% of water, respectively. Holocellulose (*ca.* 70 g.) was extracted at room temperature with 10% potassium hydroxide (1 l.) for 4 hr., and after filtration the residue was re-extracted with a further 500 ml. of alkali. The two filtrates were combined and the residue washed with water until the total volume was 2 l. Acidification of the alkaline solution with glacial acetic acid gave no precipitation but the hemicelluloses were obtained as an impure brown solid by the addition of 2 volumes of ethanol; yield 5.5 g., $[\alpha]^{23}D$ $-17.4°$ (*c* 0.52 in water). The preferred method of isolation was to add Fehling solution (200 ml.) to the alkaline extract (2 l.) and centrifuge off the green gelatinous precipitate which formed on standing overnight. The precipitate was washed with aqueous alkali and alcoholic alkali to remove excess Fehling solution, and finally with methanol. (Although the precipitated copper complex does not appear readily soluble in excess Fehling solution, an excess may inhibit its precipitation and therefore the optimum ratio of Fehling solution was determined separately.[15] A ratio of 1:10 appeared suitable). The complex was decomposed by suspending it in methanol and adding hydrochloric acid. The liberated polysaccharide was washed with methanol until free of chloride ion and then dried by solvent-exchange; yield 6.0 g. of hemicellulose I. After electrodialysis a sample had $[\alpha]^{20}D$ $-26.3°$ (*c* 1.5 in water) and equiv. wt. 1232. The supernatant from the copper complex was dialyzed against running water to remove salts and evaporated to small bulk. Acidification and addition of ethanol yielded material (0.3 g.) having $[\alpha]^{20}D$ $-31.9°$ (*c* 2 in water) which was not examined further.

Attempted Direct Extraction of Hemicelluloses from Whole Sawdust.[13]—Spruce sawdust (100 g.) was extracted for 16 hr. with hot ethanol–benzene (1:2, 900 ml.). The sawdust was washed with hot water, air-dried and then extracted at room temperature with sodium hydroxide (0.1 *N*, 900 ml.) for 48 hr., and the highly colored solution discarded. The residue was re-extracted with sodium hydroxide (1 *N*, 700 ml.) and the polysaccharide isolated *via* its copper complex; yield of hemicellulose II, 0.13 g. having $[\alpha]^{22}D$ $-13.9°$ (*c* 1.0 in water). The residue from this extraction was washed with water, delignified by the chlorite treatment and the holocellulose extracted with potassium hydroxide (10%, 1 l. and 0.5 l.). The polysaccharide isolated *via* its copper complex, yielded hemicellulose III (3.33 g.), $[\alpha]^{22}D$ $-52.6°$ (*c* 1.0 in H_2O). Samples (10 mg.) of hemicelluloses I, II and III were hydrolyzed and examined chromatographically. All showed the presence of xylose, mannose, arabinose, glucose and galactose. There were no obvious differences in the concentration of the sugars in the three samples, but no quantitative analyses were carried out.

Quantitative Analysis of Hemicellulose I.—Hemicellulose I (10 g.) was hydrolyzed with sulfuric acid (1 *N*, 360 ml.) on a steam-bath for 12 hr. The solution was neutralized ($BaCO_3$) and the filtrate passed through a column of Amberlite IR 120[16] and then through a column of Duolite A-4.[17] Evaporation of the eluate yielded the neutral sugars as a sirup (6.8 g.).

The acid fragments were eluted from the Duolite resin with sodium hydroxide (1 *N*, 50 ml.) and the eluate passed through a fresh column of Amberlite IR 120. Concentration yielded the acidic component (1.9 g.) as a hygroscopic glass, $[\alpha]^{22}D$ $+82°$ (*c* 0.5 in water).

Anal. Calcd. for $C_{12}H_{20}O_{11}$: OMe, 9.1; equiv. wt., 339. Calcd. for $C_7H_{12}O_7$: OMe, 14.7; equiv. wt., 208. Found: OMe, 10.9; equiv. wt., 274, 276.

A portion of the neutral sugars (90 mg.) was streaked on two sheets (8″ × 22″) of Whatman No. 1 paper. One sheet was developed in 1-butanol–water for 168 hr. and the other in butanone–water for 100 hr. The former solvent enabled D-xylose, D-galactose and D-glucose to be determined while the use of the latter solvent led to the determination

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of D-xylose, D-mannose and L-arabinose. Estimations were done using the phenol–sulfuric acid method.[18] The following results were obtained, using a Coleman 14 spectrophotometer: xylose, 42.1; mannose, 38.8; glucose, 8.6; galactose, 6.9; and arabinose, 4.2%.

A second portion of the neutral sugar sirup (1 g.) was added to the top of a cellulose column (40 cm. by 2.75 cm. i.d.) and the column developed with 1-butanol saturated with water at 5° at a flow rate of 9 ml./hr. Fractions were collected at 30-min. intervals.

Identification of Component Sugars.—1. Tubes 100–150 gave a trace of material having an R_f value identical with rhamnose and giving the same yellow color with *p*-anisidine trichloroacetate spray; it has not been otherwise identified. 2. D-Xylose was obtained crystalline from tubes 160–200 and had m.p. and mixed m.p. 143–145°, $[\alpha]^{22}D$ +19.4° (*c* 0.38 in water, equil.); it was further confirmed as its di-*O*-benzylidene dimethyl acetal, m.p. and mixed m.p. 208–209°. 3. D-Mannose was not obtained crystalline owing to contamination with D-xylose and L-arabinose, tubes 220–340; it was identified as its phenylhydrazone, m.p. and mixed m.p. 177–177.5°, $[\alpha]^{23}D$ +27.8 → 34.4° (93 hr., *c* 0.4 in pyridine).[19] 4. The arabinose was not separated from the mannose, tubes 230–340. The mother liquor from the mannose phenylhydrazone preparation was heated on the steam-bath for 2 hr. with Amberlite IR 120 resin to remove excess phenylhydrazine and the filtrate concentrated. Paper chromatographic examination in phenol–water (80:20 v./v.) showed a strong spot for arabinose, but treatment of the filtrate with diphenylhydrazine hydrochloride[20] failed to yield any crystalline material. An attempt was made to prepare the diphenylhydrazone from the crude neutral sirup, but this also failed. A portion of the neutral sirup, $[\alpha]^{22}D$ +32.2° (*c* 0.2 in water), was fermented for 4 days with yeast and then examined chromatographically. The presence of D-xylose, arabinose and D-galactose was demonstrated, but D-mannose was absent. It was still not possible to obtain arabinose diphenylhydrazone, but since the optical rotation was $[\alpha]^{22}D$ +41° after fermentation, it was assumed the arabinose belonged to the L-series. 5. The D-glucose sirup did not crystallize, but was identified as the N-*p*-nitrophenylglucosylamine, m.p. and mixed m.p. 172–173°.[21] 6. The D-galactose did not crystallize and an attempt to prepare the N-*p*-nitrophenylgalactosylamine yielded insufficient material to purify. Accordingly a portion of the neutral sirup was oxidized with nitric acid and the derived mucic acid had m.p. and mixed m.p. 203°.[22]

Separation of the Aldobiouronic Acid.—Chromatographic examination of the acidic fraction obtained above when developed in ethyl acetate–formic acid–acetic acid–water (18:1:3:4 v./v.) showed five distinct spots having R_x values 1.33, 0.64, 0.47, 0.38 and 0.11 (R xylose = R_x = 1.00) and several fainter ones. The value 1.33 corresponded with 4-*O*-methyl-D-glucuronic acid and that of 0.64 with 2-*O*-(4-*O*-methyl-D-glucuronosyl)-D-xylose from western hemlock.[14] The acidic mixture was accordingly fractionated on a cellulose column and the fraction having R_x 0.64 was investigated further. This fraction was slightly contamined with small amounts of fractions R_x 1.33 and R_x 0.47 and it was found later that a purer fraction could be obtained by carrying out the separation on sheets of Whatman 3 MM paper.

Methanolysis of 2-*O*-(4-*O*-Methyl-D-glucopyranosiduronic Acid)-D-xylose.—A portion of the aldobiouronic acid (198 mg.) was dissolved in methanolic hydrogen chloride (8%, 10 ml.) and sealed in a Carius tube. The tube was heated for 7 hr. at 105° and then the contents diluted with methanol and neutralized (Ag_2CO_3). Evaporation gave a dark brown sirup (157 mg.) which after two treatments with charcoal yielded a pale yellow sirup (126 mg.). This sirup was dissolved in methanol saturated with ammonia at 0° (13 ml.) and the solution kept for 24 hr. at 3°. When the solution was evaporated a clear sirup was obtained which largely crystallized in 24 hr. The crystals were washed with cold ethanol and the filtrate saved. The solid was recrystallized from ethanol containing a trace of water and yielded methyl 4-*O*-methyl-α-D-glucopyranosiduronamide, m.p. 233–236° and mixed m.p. 233–237°.[2] The ethanol washings were concentrated to a sirup (43 mg.) which was hydrolyzed with sulfuric acid (1 *N*, 4 ml.) for 12 hr. on the steam-bath. After neutralization ($BaCO_3$) and treatment with charcoal a clear sirup (18 mg.) was obtained. Since the sirup did not crystallize the di-*O*-benzylidene dimethyl acetal reagent (0.5 ml.) was added and after 24 hr. the solid was recrystallized from chloroform–petroleum ether giving D-xylose-di-*O*-benzylidene dimethyl acetal, m.p. and mixed m.p. 207–208°.[3]

Preparation and Identification of Methyl 2-*O*-(2,3,4,6-Tetra-*O*-methyl-α-D-glucopyranosyl)-3,4-di-*O*-methyl-D-xylopyranoside.—A second portion of the aldobiouronic acid (162 mg.) was converted to the ester glycoside by refluxing with 2% methanolic hydrogen chloride. After neutralization (Ag_2CO_3) the glycoside was reduced by refluxing in tetrahydrofuran with lithium aluminum hydride (0.5 g.) for 1 hr. The reduced product was isolated *via* its acetate and deacetylated as previously described.[15] The neutral disaccharide (153 mg.) was dissolved in water (6 ml.) to which was added sodium hydroxide (1.5 g.). Dimethyl sulfate (1.5 ml.) was added over 3.5 hr. with vigorous stirring. During the final half-hour the temperature was raised to 70° to hydrolyze excess dimethyl sulfate. The cold methylation solution was brought to *p*H 8.9 with sulfuric acid and continuously extracted with chloroform overnight. Concentration of the chloroform yielded a sirup (88 mg.). This sirup together with 175 mg. from a previous experiment was dissolved in tetrahydrofuran (12 ml.) to which was added pulverized sodium hydroxide (2.3 g.). Dimethyl sulfate (3.0 ml.) was added to the vigorously stirred solution over 3 hr. and the reaction stirred at room temperature for a further 16 hr. Sufficient water was added to dissolve the salts, the solution heated to 60° for 1 hr., cooled and extracted with chloroform.[23] The fully methylated disaccharide was obtained as a pale yellow sirup (111 mg.), b.p. (bath temp.) 125–150° (0.08 mm.), and was hydrolyzed with sulfuric acid (1 *N*, 10 ml.) for 12 hr. on the steam-bath. The acid was neutralized by passage through Duolite A-4 and the eluate concentrated to a sirup (120 mg.). Chromatographic examination in butanone–water azeotrope showed only two spots corresponding to tetra-*O*-methylhexose and di-*O*-methylpentose. The sirup was streaked on Whatman No. 3MM paper (18″ × 22″) and developed in the same solvent. The appropriate zones were eluted with a mixture of acetone and methanol (50:50 v./v.). Concentration of one solution gave 2,3,4,6-tetra-*O*-methyl-D-glucose which crystallized spontaneously and was recrystallized from ether, m.p. and mixed m.p. 92–93°. The other solution gave a sirup (22 mg.) having $[\alpha]^{21}D$ +15° (*c* 0.16 in methanol); lit. values for 3,4-di-*O*-methyl-D-xylose +13°[9] and +22.1°[24] in methanol. The sirup was dissolved in water (1 ml.) and excess bromine added. The solution was kept in the dark at room temperature in a tightly stoppered flask for two days and the excess bromine removed by aeration. The solution was neutralized with silver carbonate, filtered and the residual silver removed with hydrogen sulfide. The final sirup was distilled, b.p. (bath temp.) 150–170° (0.07 mm.), and yielded a clear sirup (12 mg.) which crystallized on nucleation. The 3,4-di-*O*-methyl-D-xylono-δ-lactone was recrystallized from ether and had m.p. and mixed m.p. 61–64°. (We are grateful to Dr. J. K. N. Jones for an authentic sample.)

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