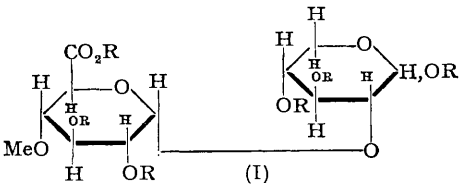
651. *The Hemicelluloses present in Aspen Wood* (Populus tremuloides). *Part II.**

By J. K. N. Jones and Louis E. Wise.

Fractionation of the uronic acids produced on hydrolysis of aspen sawdust has yielded D-galacturonic acid and 2-α-(4-methyl D-glucuronosyl) α-D-xylose as well as several unidentified acidic fractions of higher molecular weight.

In Part I,* the isolation from the hydrolysis products of aspen wood of 4-methyl D-glucuronic acid and its identification were described. In addition, evidence was given for the presence of other uronic acid-containing materials. Two of these have been identified as D-galacturonic acid and as 2-α-(4-methyl D-glucuronosyl) D-xylose (I; R = H). The presence of D-galacturonic acid was proved by its oxidation to mucic acid and its conversion into the methyl ester of α-methyl-D-galacturonoside and into 2 : 3 : 4 : 6-tetramethyl *N*-phenyl-D-galactosylamine. The identification of this acid confirms the work of Anderson and Wise (*Paper Ind. Paper World*, 1945, **27**, No. 7, 1037).

The identification of the aldobiuronic acid depends on the following evidence. On hydrolysis, which required very drastic conditions and caused extensive degradation, D-xylose and 4-methyl D-glucuronic acid were produced. Methylation of the aldobiuronic acid with sodium hydroxide and methyl sulphate, followed by methylation with Purdie's reagents, yielded the fully methylated derivative (I; R = Me). This material was very resistant to hydrolysis by aqueous acids, but on long boiling was cleaved with extensive decomposition, yielding 2 : 3 : 4-trimethyl D-glucuronic acid, identified as the amide of the corresponding α-methylglycoside. 3 : 4-Dimethyl D-xylose was also produced and was characterised by its rate of movement on the chromatogram and by its conversion into a crystalline aniline derivative and a lactone which had properties closely similar to those of 3 : 4-dimethyl D-xylonolactone (James and Smith, *J.*, 1945, 744). The methylated aldobiuronic acid was hydrolysed with less decomposition if subjected first to methanolysis and then to hydrolysis by aqueous acids.



(I)

When (I; R = Me) was reduced with lithium aluminium hydride, a glucosyl xylose derivative was produced which, on methylation with Purdie's reagents, yielded 3 : 4-dimethyl 2-(2 : 3 : 4 : 6-tetramethyl D-glucosyl) methylxyloside. The sugar residues were probably joined by an α-linkage as this material possessed a high positive rotation. On hydrolysis with dilute aqueous acids, better yields of the xylose derivative were produced (compare above). The methylated xylose and methylated glucose were separated by chromatography on filter paper, and characterised as the crystalline aniline derivative and lactone and as the crystalline sugar and aniline derivative respectively. The lactone, on reaction with ammonia, gave a syrupy amide which gave a positive Weerman reaction (cf. James and Smith, *loc. cit.*). The dimethyl sugar (which is differentiated from

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2 : 3- and 2 : 4-dimethylxylose on the chromatogram), on methylation with Purdie's reagents, followed by hydrolysis with dilute acid, yielded 2 : 3 : 4-trimethyl D-xylose. These facts prove that the derivative of xylose is the 3 : 4-dimethyl compound.

A fraction containing both the aldobiuronic acid and D-galacturonic acid was oxidised with bromine water and the product methylated with sodium hydroxide and methyl sulphate. Isolation of the methylated acidic material and further methylation led to the crystalline dimethyl ester of 2 : 3 : 4 : 5-tetramethyl galactosaccharate. This provides further proof of the presence of galacturonic acid in the mixture of acids produced on hydrolysis of aspen wood. No derivative of xylonic acid could be isolated.

Galacturonic acid and the (4-methyl glucuronosyl) xylose move at the rate of galactose and glucose on the paper chromatogram when solvent C (see Experimental section) is employed. Examination of the mixture of uronic acids produced on hydrolysis of aspen sawdust showed that several other uronic acid derivatives which moved at slower rates were also present. On hydrolysis, they gave a mixture of sugars provisionally identified as galactose, xylose, rhamnose, and glucuronic and 4-methyl glucuronic acids. The origin of these acids and of (I; R = H) and of the galacturonic acid is uncertain, but it is possible that the former is produced by hydrolysis of xylan and the latter from pectic acid (cf. Anderson and Wise, *loc. cit.*).

Experimental

The following solvents (vol./vol.) were used to separate the sugars and their derivatives: (A) butanol–ethanol–water (40 : 11 : 19), (B) butanol–pyridine–water (10 : 3 : 3), (C) ethyl acetate–acetic acid–formic acid–water (18 : 3 : 1 : 4), and (D) ethanol–benzene–water (47 : 169 : 15; top layer). The R_G values quoted are given to show the positions of the sugars on the chromatogram relative to 2 : 3 : 4 : 6-tetramethyl glucose; they are not absolute values.

Optical rotations were observed at 20°.

The acids were prepared by hydrolysis of aspen wood with hot N-sulphuric acid and were separated, first on columns of charcoal and then by elution from columns of cellulose (see Part I, which describes Fraction BII).

Identification of 2-(4-*Methyl* D-*Glucuronosyl*) D-*Xylose*.—Fraction BII (0·54 g.) was boiled under reflux with methanolic hydrogen chloride (4% w/v; 50 c.c.) for 24 hours. The solution was then evaporated to 10 c.c., N-hydrochloric acid (40 c.c.) added, and the solution boiled for a further 20 hours. The cooled solution was neutralised with silver carbonate and filtered, and the silver ions were removed from the filtrate by hydrogen sulphide. The filtered solution was concentrated to 5 c.c., and a portion examined on the chromatogram with solvent C on the Whatman No. 1 paper. Xylose, 4-methyl glucuronic acid, and unchanged aldobiuronic acid were detected. The remaining solution was evaporated to a syrup which was separated into its components by chromatography on two large sheets of filter paper (solvent C). Extraction of the appropriate strips of paper gave D-xylose, m. p. and mixed m. p. 144°, $[\alpha]_D$ +18° (equil. in water) and 4-methyl D-glucuronic acid, $[\alpha]_D$ +80° (*c*, 0·6 in water) (Found: OMe, 13·3; equiv., 210. Calc. for $C_7H_{12}O_7$: OMe, 14·9%; equiv., 208).

Methylation.—The aldobiuronic acid (0·9 g.), dissolved in water, was exactly neutralised with barium hydroxide. The filtered solution was evaporated to dryness and the residual barium salt (1·04 g.) (Found: OMe, 5·4; Ba, 16·0. Calc. for $C_{24}H_{38}O_{22}Ba$: OMe, 7·6; Ba, 16·8%. 15·4 mg. gave, on distillation with phosphoric acid, furfuraldehyde equivalent to 6 mg. of xylose) was methylated by dissolving it in water (5 c.c.) and adding methyl sulphate (2 c.c.) and then sodium hydroxide (30%; 5 c.c.) dropwise with vigorous stirring and cooling. When the solution no longer reduced Fehling's solution (24 hours), an excess of 30% sodium hydroxide (10 c.c.) was added and methyl sulphate (5 c.c.) added dropwise with stirring during 24 hours. The solution was then heated on the boiling-water bath for 30 minutes, cooled, acidified with 2N-sulphuric acid, and extracted continuously with chloroform. Concentration of the extract yielded a syrup (1·0 g.) which was further methylated with silver oxide and methyl iodide and distilled [yield, 0·72 g.; b. p. 190° (bath-temp.)/0·01 mm.; n_D^{19} 1·4638; $[\alpha]_D^{20}$ +101° (in water, *c*, 0·3)] (Found: OMe, 48·5. Calc. for $C_{18}H_{32}O_{11}$: OMe, 51·3%). This methylated ester glycoside was very difficult to hydrolyse: after 24 hours in boiling 2N-sulphuric acid, less than half had been hydrolysed and the products had suffered extensive decomposition with formation of furfuraldehyde. Accordingly, it (0·32 g.) was boiled with methanolic hydrogen chloride (5% w/v; 25 c.c.) for 48 hours, the solution then evaporated to 5 c.c., N-hydrochloric acid

(20 c.c.) added, and the solution boiled for a further day. The cooled solution was neutralised with barium hydroxide, concentrated to 10 c.c., and exhaustively extracted with chloroform, thus giving 3 : 4-dimethyl D-xylose (111 mg.). The aqueous solution was then acidified with sulphuric acid and exhaustively extracted with chloroform to give 2 : 3 : 4-trimethyl D-glucuronic acid (130 mg.).

Identification of 3 : 4-*Dimethyl* D-*Xylose.*—(a) This sugar showed $[\alpha]_D^{20}$ +31°±5° (*c*, 0·57 in methyl alcohol). In solvent (A), it had R_G 0·74 and gave a reddish spot, whereas the 2 : 4- and the 2 : 3-isomer had R_G 0·73 and 0·85 respectively and gave spots of a purplish colour with the *p*-anisidine hydrochloride spray. In solvent (B), the R_G values were 0·85 for the 3 : 4-isomer and 0·84 for the 2 : 4-isomer, in solvent (C) 0·78 and 0·75, and in solvent (D) 0·47 and 0·31 respectively.

(b) *Oxidation with metaperiodate.* The sugar (29 mg.) was dissolved in water (4 c.c.) and oxidised with 0·25M-sodium metaperiodate solution (1 c.c.). Initially, the sugar could be detected on the paper chromatogram, but after 24 hours it was no longer detectable and a new sugar derivative appeared, which moved faster and gave a different colour (brown instead of red) with the *p*-anisidine hydrochloride spray. Ethylene glycol was added to the reaction mixture and the liberated acid titrated with 0·02N-sodium hydroxide (Found : 3·5 c.c. Calc. : 8·1 c.c.). Further quantities of acid (1·1 c.c.; 0·02N) were later slowly produced (from the hydrolysis of a formyl ester ?).

(c) *Oxidation with bromine water.* The sugar (61 mg.) was oxidised with bromine water (5 c.c.) for 48 hours, whereafter it could no longer be detected as such on the paper chromatogram (solvent B). Bromine was removed by aëration and the solution neutralised with silver carbonate and filtered. Silver ions were removed from the filtrate by passage of hydrogen sulphide, whereupon the solution was again filtered. Concentration of this filtrate yielded 3 : 4-dimethyl D-xylonolactone (51 mg.) which crystallised spontaneously. It was purified by distillation and had b. p. 120° (bath-temp.)/0·1 mm., m. p. 67° (from ether)((Found : OMe, 34·0. Calc. for $C_7H_{12}O_5$: OMe, 35·2%). The lactone mutarotates at the rate of a pyranolactone in aqueous solution : $[\alpha]_D^{20}$ (*c*, 2·0 in water), −51° (30 minutes); −46° (7 hours); −35° (23½ hours); −23° (49 hours; constant). James and Smith (*loc. cit.*) record m. p. 68°, $[\alpha]_D^{18}$ −54° ⟶ −27° (65 hours) for this compound.

A portion of the lactone (8 mg.) with methanolic ammonia gave a syrupy amide (8 mg.). On oxidation with sodium hypochlorite under the conditions described by James and Smith (*loc. cit.*), this gave sodium cyanate, detected as hydrazodicarbonamide (2 mg.), m. p. 258° (decomp).

Identification of the Uronic Acid Fraction.—(a) The uronic acid fraction (111 mg.) was found on the chromatogram (solvent C) to consist of 2 : 3 : 4-trimethyl D-glucuronic acid (mainly) and 3 : 4-dimethyl 2-α-(2 : 3 : 4-trimethyl D-glucuronosyl) D-xylose (?) (see below). On boiling with methanolic hydrogen chloride (25 c.c.; 1% w/v), esterification and glycoside formation took place simultaneously, with the production mainly of the methyl ester of 2 : 3 : 4-trimethyl α-methyl-D-glucuronoside. This was isolated as a syrup after neutralisation of the cooled methanolic solution with silver carbonate and concentration of the filtrate. When the crude ester glycoside was dissolved in methanol and the solution saturated with ammonia, the corresponding amide (10 mg.) was produced, having m. p. and mixed m. p. 188° (from acetone–light petroleum), $[\alpha]_D$ +150° (*c*, 0·1 in water). The residual crude amide was boiled with methanolic hydrogen chloride (25 c.c.; 3% w/v), and the crude ester isolated as described above. This was reduced to the glucose derivative by addition of the ester in ether to a solution of lithium aluminium hydride (0·5 g.) in ether. Excess of the reagent was destroyed after 15 minutes, by addition of ethyl acetate. The solution was filtered and the filtrate and washings were deionised with Amberlite IR-400 and IR-120, and evaporated to a syrup (80 mg.). Examination on the chromatogram of the products of hydrolysis (solvent A) of a portion of this fraction showed the presence of a dimethyl pentose and of a trimethyl hexose derivative. The syrup was methylated (Purdie's reagents) and hydrolysed with N-hydrochloric acid. The sugars (50 mg.), isolated in the usual way, were found on the chromatogram (solvent D) to consist of 3 : 4-dimethyl D-xylose, 2 : 3 : 4-trimethyl D-xylose and 2 : 3 : 4 : 6-tetramethyl D-glucose, the last greatly predominating. The sugars were separated on a sheet of filter paper (Whatman No. 1) (solvent A). After extraction of the appropriate section of the chromatogram, tetramethyl D-glucose (28 mg.) was obtained, having m. p. and mixed m. p. 90° with previous sintering at 86°. The aniline derivative prepared from this material in the usual manner had m. p. 114° on recrystallisation from light petroleum (b. p. 40—60°), raised to 138° on recrystallisation from alcohol, and $[\alpha]_D^{20}$ was +200° (*c*, 0·5 in acetone). A specimen of tetramethyl *N*-phenylglucosylamine was, therefore, prepared from authentic material and found to possess m. p. 114° on

recrystallisation from light petroleum (b. p. 40—60°), not depressed on admixture with the above sample, but raised to 138° on recrystallisation from alcohol.

(b) *Oxidiation with metaperiodate at 2°.* The barium salt (122 mg.) was dissolved in water (5 c.c.), and sodium sulphate (100 mg.) added, followed by 0·1M-sodium metaperiodate (20 c.c.). At intervals, portions of the solution (2 c.c.) were withdrawn, ethylene glycol added, and the liberated formic acid titrated [Found: c.c. of 0·01N-formic acid per 122 mg. of barium salt, 6·3 (½ hour), 11·2 (1½ hours), 18 (3·1 hours), 26·2 (6 hours), 34·2 (7½ hours), 86·2 (23 hours), 102·4 (70 hours); iodine was liberated after 100 hours].

(c) *Oxidation with bromine water.* The barium salt (1·05 g.) was dissolved in water (10 c.c.), and barium benzoate (1·6 g.) and bromine (2 c.c.) were added. After 48 hours, the solution failed to reduce Fehling's solution. Bromine was removed by aëration, benzoic acid was filtered off, and the solution concentrated to dryness. The residue (2·7 g.) was methylated with sodium hydroxide and methyl sulphate in the usual manner. Addition of the sodium hydroxide solution caused the reaction mixture to develop a dark orange colour. After addition of methyl sulphate (10 c.c.) and sodium hydroxide (20 c.c.; 30%), the solution was heated on the boiling-water bath for 30 minutes, cooled, acidified with 2N-sulphuric acid, and extracted exhaustively with chloroform. Concentration of the extracts gave a syrup (0·29 g.) which contained crystals. This was difficult to purify; accordingly, it was methylated with Purdie's reagents and the product (0·29 g.) distilled. The product, the dimethyl ester of 2 : 3 : 4 : 5-tetramethyl galactosaccharate (0·15 g.), b. p. 120° (bath-temp.)/0·1 mm., crystallised. Recrystallised from light petroleum (b. p. 60—80°), it had m. p. 104° (Found: OMe, 61·1; equiv., 146. Calc. for $C_{12}H_{22}O_8$: OMe, 63·3%; equiv., 147).

Methylation of Fraction BID (cf. Part I).—This acidic fraction {1·3 g.; $[\alpha]_D^{20}$ +51° (*c*, 13·0 in water)} was titrated with barium hydroxide and the neutral solution evaporated to dryness. The barium salt (1·38 g.) (Found: Ba, 16·0; OMe, 5·4%) was observed on the chromatogram (solvent C) to contain two components, one moving at the rate of glucose and the other at the rate of galactose. The sugars did not move in solvent B. The mixture of barium salts (1·3 g.) was dissolved in water (10 c.c.) and methylated by the addition of methyl sulphate (10 c.c.) and sodium hydroxide (30%) dropwise, with vigorous stirring. After 12 hours, an excess of 30% sodium hydroxide (20 c.c.) was added and the methylation continued by portion-wise addition of the methyl sulphate. After 48 hours, the solution was heated on the boiling-water bath for 30 minutes, cooled, acidified with 2N-sulphuric acid, and extracted exhaustively with chloroform. Concentration of the extracts gave a syrup (1·12 g.) which was further methylated with Purdie's reagents. The resulting neutral syrup was reduced with lithium aluminium hydride (2 g.) in ether during 30 minutes. Excess of the reagent was destroyed with ethyl acetate, and water was then added to the solution. The slurry was filtered and the precipitate well washed with water. The combined alkaline filtrates were concentrated to *ca.* 10 c.c. and exhaustively extracted with chloroform. The syrup (0·91 g.) resulting from concentration of the chloroform solution was further methylated with silver oxide and methyl iodide, and the resulting syrup (0·90 g.) distilled, giving: fraction I (0·21 g.), mainly tetramethyl methyl D-galactoside, b. p. 120° (bath-temp.)/0·1 mm., n_D^{18} 1·4495 (Found: OMe, 60·8. Calc. for $C_{11}H_{22}O_6$: OMe, 62%); fraction II (0·57 g.), methylated glucosidyl xylose, b. p. 200° (bath-temp.)/0·1 mm. (Found: OMe, 51·6. Calc. for $C_{18}H_{34}O_{10}$: OMe, 52·9%).

Fraction I (0·2 g.) was hydrolysed with boiling N-sulphuric acid (10 c.c.) {$[\alpha]_D^{20}$ +50° ⟶ +70° (constant) in 5 hours}. The solution was neutralised with barium hydroxide and exhaustively extracted with chloroform. Concentration of the extract gave a syrupy mixture of sugars (0·188 g.), n_D^{20} 1·4600, $[\alpha]_D^{20}$ +75° (*c*, 1·88 in water). Examination on the paper chromatogram (solvent A) indicated the presence of tetramethyl galactose and traces of tetramethyl glucose and trimethyl xylose. Accordingly, the syrup was boiled with alcoholic aniline for 2 hours. Concentration then gave 2 : 3 : 4 : 6-tetramethyl *N*-phenyl-D-galactosylamine, m. p. and mixed m. p. 191°, $[\alpha]_D^{20}$ −80° ⟶ +40° (*c*, 0·3 in acetone), which moved at the same rate on the chromatogram as did an authentic sample (solvent B). A small quantity of the aniline derivative dissolved in N-hydrochloric acid gave 2 : 3 : 4 : 6-tetramethyl galactose which moved at the same rate in solvent (B) as did the sugar from an authentic sample of aniline derivative which had been treated similarly. No other crystalline aniline derivative was isolated.

Fraction II (0·55 g.) was hydrolysed with boiling N-sulphuric acid (10 c.c.) for 13 hours {$[\alpha]_D^{20}$ +104° ⟶ +47° (constant) (*c*, 5·5 in N-sulphuric acid)}. The cooled solution was neutralised with barium hydroxide and exhaustively extracted with chloroform, yielding a syrup (0·60 g.). This gave spots on the chromatogram (solvent C) corresponding to 2 : 3 : 4 : 6-tetramethyl glucose, 3 : 4-dimethyl xylose, and traces of trimethyl hexose and monomethyl

pentose. The sugars were separated on paper sheet chromatograms, solvent (A) being used to irrigate the papers. Concentration of the appropriate sections of paper gave 2 : 3 : 4 : 6-tetramethyl D-glucose (0·32 g.), m. p. and mixed m. p. 93°, $[\alpha]_D^{20}$ +83° (*c*, 0·26 in water) (aniline derivative, m. p. and mixed m. p. 138°), and syrupy 3 : 4-dimethyl D-xylose (0·16 g.), characterised as its crystalline lactone, m. p. and mixed m. p. 67°, and as its aniline *derivative* (prepared in the usual manner), m. p. 121° (R_G 1·08 in solvent B) (Found : C, 61·5; H, 7·8; N, 5·5. $C_{13}H_{19}O_4N$ requires C, 61·7; H, 7·6; N, 5·5; OMe, 24·5%). The aniline derivative is unstable and darkens rapidly in air.

Methylation of 3 : 4-*Dimethyl Xylose.*—The sugar (25 mg.) was methylated with Purdie's reagents, and the product (20 mg.) isolated in the usual way. The syrup, n_D^{20} 1·4440, was hydrolysed with N-sulphuric acid (5 c.c.) on the boiling-water bath for 5 hours and the sugar isolated by continuous extraction of the neutralised solution (sodium hydroxide) with chloroform. The sugar (16 mg.) had m. p. 91°, not depressed on admixture with an authentic specimen of 2 : 3 : 4-trimethyl D-xylose.

Fraction BIE.—Identification of D-*galacturonic acid.* This fraction, $[\alpha]_D^{20}$ +63° (*c*, 7·0 in water), was neutralised with barium hydroxide, and the isolated barium salt examined (Found: Ba, 21·2; OMe, 1·5%. Calc. for the barium salt of galacturonic acid : Ba, 26·7%). A sample of the barium salt (100 mg.) was warmed with bromine water for 3 hours. White crystals separated. They were filtered off and washed with water, alcohol, and ether [yield, 21 mg.; m. p. 221° (decomp.), not depressed on admixture with mucic acid (cf. Anderson and Wise, *loc. cit.*)]. A further sample of the barium salt (0·7 g.) was dissolved in water, and barium ions were removed with Amberlite resin IR-120. The acidic solution was concentrated to a syrup which was boiled with methanolic hydrogen chloride (1%; 50 c.c.) for 12 hours. The cooled solution was neutralised with silver carbonate and filtered, and the filtrate concentrated to a syrup (0·7 g.) which crystallised. The crystals were filtered off and recrystallised from moist acetone, yielding the hydrate of the methyl ester of α-methyl-D-galacturonoside, m. p. and mixed m. p. 143° (0·2 g.), $[\alpha]_D^{20}$ +124° (*c*, 0·2 in water). This material moved at the same rate as did rhamnose on the paper chromatogram in solvent (B). The syrupy residue contained at least five components, four of which moved faster than did the above derivative of galacturonic acid on the chromatogram in solvent (B).

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THE INSTITUTE OF PAPER CHEMISTRY,
APPLETON, WISCONSIN, U.S.A.
THE UNIVERSITY, BRISTOL, ENGLAND.

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