903. *The Biosynthesis of Polysaccharides. Part I. The Composition of Plum-leaf Polysaccharides.*

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Hydrolysis of the polysaccharides from Victoria plum leaf afforded mainly D-galactose, D-glucose, L-arabinose, D-xylose, and D-galacturonic acid. Smaller amounts of D-mannose, L-rhamnose, L-fucose, 2-*O*-methyl-D-xylose, 2-*O*-methyl-L-fucose, and glucuronic acid were also isolated. Some fractionation of the leaf polysaccharides was achieved by successive extractions with water and aqueous alkalis. The cellulose content has been investigated.

PRELIMINARY results [1] on the incorporation of radioactive carbon into the components of plum-leaf polysaccharides as a result of photosynthesis in $^{14}CO_2$, suggested that after 48 hours the isotope was present in all the major monosaccharide constituents. In order to further these biosynthetic studies [2] it was necessary to characterise firmly the monosaccharide components and to investigate the fractionation of the polysaccharides.

Mature Victoria plum leaves were exhaustively extracted with methanol, and the insoluble residue, containing the polysaccharides, was heated with N-sulphuric acid for several short periods. From the combined hydrolysates were isolated three fractions, a mixture of neutral monosaccharides, small amounts of acidic oligosaccharides, and a degraded pectic acid fraction. The last was recognised by hydrolysis with a pectinase preparation [3] and with acid to give D-galacturonic acid which was characterised *via* the derived methyl ester methyl glycoside by reduction with borohydride to D-galactose.

Examination of a hydrolysate of the acidic oligosaccharide mixture by paper chromatography indicated the presence of traces of acidic oligosaccharides, together with glucurono-6 ⟶ 3-lactone, mono-*O*-methylhexuronic acid, glucuronic acid, and galacturonic acid. The last two hexuronic acids were recognised by reduction as above to glucose and galactose and isolation of D-galactose.

Fractionation of the neutral monosaccharides by formation of di-*O*-*iso*propylidene derivatives and subsequent selective hydrolysis [4] was not entirely satisfactory in view of the losses incurred, although D-glucose and D-xylose, and D-galactose and L-arabinose, were considerably enriched in separate fractions. Liberation of the free monosaccharides from

[1] Hough and Pridham, *Nature*, 1956, **177**, 1039.
[2] Andrews and Hough, *Biochem. J.*, 1957, **67**, 11P.
[3] Reid, *J. Sci. Food Agric.*, 1950, **1**, 234.
[4] Bell, *J.*, 1947, 1461.

the mixed *O-iso*propylidene derivatives by acid hydrolysis, followed by paper-chromatographic separations, eventually afforded crystalline specimens of D-galactose, D-glucose, L-arabinose, D-xylose, and L-rhamnose. D-Mannose was isolated as a syrup and characterised by conversion into its phenylhydrazone. Further hydrolysates of the leaf polysaccharides yielded sufficient L-fucose for subsequent characterisation.

Traces of unidentified reducing sugars which moved ahead of rhamnose on the paper chromatogram were also detected in the neutral sugar mixture. Adsorption of the mixture on a squat charcoal column [5] followed by fractional elution with ethanol–water afforded 2-*O*-methyl-D-xylose [6] and 2-*O*-methyl-L-fucose.[7] These *O*-methyl sugars have also been isolated from the alkali-stable component (" araban ") of sugar-beet pectin.[8] Springer, Ansell, and Ruelius [9] have obtained evidence, by paper chromatography and serological tests, for the presence of 2-*O*-methylfucose in the polysaccharides of yew twigs (*Taxus cuspidata*), and 2-*O*-methyl-xylose and -arabinose are reported to occur in Delaware soils.[10] These monosaccharides are unique in that they afford the only known instances of *O*-methylaldopentoses and of 2-*O*-methyl substitution amongst naturally occurring carbohydrates. Preliminary examination has now indicated that these *O*-methyl sugars are minor constituents of the polysaccharides of various leaves (*e.g.*, bean and clover) and of citrus pectins.

The relatively large number of monosaccharide constituents found in the leaf polysaccharides indicated that the latter formed a mixture of some complexity. This was corroborated by exhaustive extraction of the alcohol-insoluble leaf residue with cold water, hot water, cold N-sodium hydroxide, and hot 2·5N-sodium hydroxide in succession, and the subsequent isolation of a number of polysaccharide fractions (see Table). In general, each extract contained similar monosaccharide constituents, but in different relative proportions. The water-soluble polysaccharides resembled pectin, with galactose, arabinose, and galacturonic acid as the major constituents. Rhamnose formed a higher proportion (10—15%) of the cold-water fraction than of any other fractions; on the other hand glucose, a constituent of all the other fractions, was absent. In comparison, the fraction soluble in hot water was richer in arabinose, and the presence of xylose was noted. The polysaccharides extracted with alkali contained markedly increased proportions of glucose and xylose, and arabinose was still a major constituent, but galactose now formed only a small part of the total. The alkali-insoluble residue still contained the pentoses as well as glucose. The material remaining after treatment with acid chlorite contained starch and cellulose, the latter representing only a very small proportion (<1%) of the original leaf residue. An estimate of the cellulose content of the leaf residue, by Crampton and Maynard's method,[11] gave a value of 8·5%, but further examination of the cellulosic material obtained by this method indicated that the true value was of the order of 5%.

The wide variety of monosaccharides which occurs in the leaf accounts for all the monosaccharides found in other organs of the plant, including the fruit.[12] This suggests that the monosaccharides have their origin within the leaf structure and are initially incorporated into the attendant polysaccharides,[1,2] but that translocation to other parts of the plant might follow in due course.

Experimental

Partition chromatography, by the descending method, was carried out on either Whatman No. 1 or No. 540 filter paper, with one of the following solvent systems: (*a*) ethyl acetate–acetic acid–water (9 : 2 : 2 v/v); (*b*) butan-1-ol–pyridine–water (10 : 3 : 3 v/v); (*c*) butan-1-ol–ethanol–water (40 : 11 : 19 v/v). Sugars were detected on the chromatograms with *p*-anisidine

[5] Andrews, Hough, and Powell, *Chem. and Ind.*, 1956, 658.
[6] Andrews and Hough, *ibid.*, 1956, 1278.
[7] Anderson, Andrews, and Hough, *ibid.*, 1957, 1453.
[8] Andrews, Hough, Powell, and Woods, unpublished results.
[9] Springer, Ansell, and Ruelius, *Naturwiss.*, 1956, **11**, 256.
[10] Lynch, Olney, and Wright, *J. Sci. Food Agric.*, 1958, **9**, 56.
[11] Crampton and Maynard, *J. Nutrition*, 1938, **15**, 383.
[12] Pridham, Ph.D. Thesis, Bristol, 1955.

hydrochloride,[13] and the figures given for the relative amounts of monosaccharides in hydrolysates were obtained by visual comparison of their spot sizes; R_{Rh} is the rate of movement of a sugar on the chromatogram relative to rhamnose. Unless otherwise stated, hydrolyses were carried out in N-sulphuric acid at 100°, and evaporations were at 40° under reduced pressure. Amberlite ion-exchange resins were used throughout, and optical rotations were determined in water at 20°.

Plum leaves (*Prunus domestica* var. Victoria) were picked during August, and immediately immersed in alcohol. After removal of the stems, the leaves were continuously extracted with methanol in a Soxhlet apparatus. The alcohol-insoluble material was air-dried at 60°, and coarsely powdered (yield, about 15% w/w of fresh leaves) (Found: Moisture, 9·7%). Evaporation of the alcoholic extracts gave a dark solid (yield, about 8% w/w).

Hydrolysis of Alcohol-insoluble Leaf Residue.—A small portion of this material was hydrolysed for 16 hr. and after neutralisation with barium carbonate the hydrolysate was found by paper chromatography to contain galactose, glucose, arabinose, xylose, and hexuronic acid (*ca.* 1 : 2 : 2 : 1 : 1), with a smaller amount of rhamnose and traces of a compound with R_{Rh} 1·25 in solvent (*b*), which gave a pink colour with *p*-anisidine hydrochloride.

For isolation of the monosaccharides, alcohol-insoluble leaf residue (37 g.) was hydrolysed with acid (300 c.c.) for 4 hr., then the insoluble material was isolated on the centrifuge and again heated with acid (2 × 250 c.c.; 3 hr. each time). The final residue (R) was washed with hot water until the washings contained no carbohydrate (Molisch test), and was retained. The red, acid hydrolysates and the washings were combined (1·5 l.), and barium carbonate (70 g.) was added with stirring. When reaction had ceased, the mixture was centrifuged and the supernatant liquor (*ca.* pH 2) evaporated to *ca.* 500 c.c. Addition of ethanol (1 l.) produced a white precipitate (P) which was isolated on the centrifuge, then reprecipitated with ethanol from aqueous solution, washed with ethanol and ether, and dried (1·2 g.).

The supernatant liquor from the isolation of (P) was concentrated to *ca.* 400 c.c. and neutralised with barium carbonate. The mixture was centrifuged, and evaporation of the supernatant liquor gave a neutral brown syrup (10·6 g.), in which the following reducing sugars were detected by paper chromatography: galactose, glucose, arabinose, xylose (main components), rhamnose, fucose, compounds with R_{Rh} 1·25 and 1·31 in (*b*) [(A) and (B) respectively, see below], hexuronic acid, and oligosaccharides (minor components). Considerable amounts of amino-acids (located with ninhydrin) were also present.

Residue (R) was further heated with acid (2 × 250 c.c.; 3 hr. each time), but addition of ethanol (300 c.c.) to the concentrated hydrolysate (150 c.c.) produced only a slight precipitate, which was not further examined. After evaporation of the ethanol, the solution was neutralised as before, the solids were removed on the centrifuge, and the supernatant liquor was concentrated. The resultant syrup (0·6 g.) contained xylose (mainly), with galactose, glucose, arabinose, and acidic oligosaccharides.

An aqueous solution of the combined monosaccharide-containing syrups was passed successively through columns of IR-120(H) and IRA-400(CO_3) resin, then the column effluent, after concentration to *ca.* 200 c.c., was allowed to percolate through a squat charcoal column [5] (diam. 10 cm., length 4 cm.) which had been previously washed with ethanol and water. Elution of the column with water (1·5 l.) afforded a fraction (3·3 g.) containing galactose, glucose, arabinose, xylose, and rhamnose, and much smaller amounts of fucose and mannose. Further elution with alcohol–water gave mixtures of compounds (A) and (B), and of oligosaccharides (0·8 g.). Hydrolysis of the latter yielded a mixture (0·66 g.) consisting mainly of galactose, glucose, arabinose, and xylose.

Examination of Acidic Mono- and Oligo-saccharides.—Treatment of the IRA-400(CO_3) resin used in the above purification with excess of N-sulphuric acid, followed by washing with water, gave a solution which after neutralisation (as above), filtration, and evaporation yielded a white solid (2·7 g.) which apparently contained small amounts of at least three aldobiouronic acids, with higher oligosaccharides. Cations were removed from the mixture on IR-120(H) resin, and the remaining material hydrolysed with 2N-acid. Passage of the hydrolysate through an IR-4B(OH) resin column and evaporation of the effluent gave a syrup (0·2 g.) containing galactose, glucose, xylose, and rhamnose. The resin column was then eluted with 0·1N-sulphuric acid until carbohydrate was no longer displaced (Molisch test). The acid effluent was neutralised as before, filtered, passed through IR-120(H) resin, and evaporated. The resultant syrup

[13] Hough, Jones, and Wadman, *J.*, 1956, 1702.

(0·2 g.) consisted mainly of hexuronic acid and acidic oligosaccharides, but glucurono-6 ⟶ 3-lactone and mono-*O*-methylhexuronic acid (both recognised by chromatographic behaviour) were also present. Chromatographic separation on paper sheets with solvent (*a*) gave hexuronic acid (30 mg.), a mixture of acidic oligosaccharides (80 mg.), glucurono-6 ⟶ 3-lactone (<5 mg.), and mono-*O*-methylhexuronic acid (<5 mg.). Hydrolysis of the acidic oligosaccharides with 2N-acid for 18 hr. gave rhamnose, galactose, and hexuronic acid; glucurono-6 ⟶ 3-lactone was not detected.

The hexuronic acid fraction (30 mg.) was converted into methyl ester methyl glycoside by heating it under reflux for 18 hr. in methanolic hydrogen chloride (2% w/w), and the isolated product was reduced with potassium borohydride (20 mg.) in water (2 c.c.). After 2 hr., 2N-sulphuric acid (3 c.c.) was added, then the solution was heated at 100° for 6 hr., passed through IR-120(H) and IRA-400(CO_3) resins, and evaporated, yielding a syrup (12 mg.) containing galactose and glucose (*ca.* 3 : 1). Crystallisation from methanol gave D-galactose, m. p. and mixed m. p. 162—164°.

Examination of Precipitate (*P*).—After drying, this material (1·2 g.) dissolved only partially in water, giving a solution with an acid reaction. Hydrolysis (16 hr.), followed by paper chromatography, indicated that hexuronic acid was the main monosaccharide constituent, but xylose and traces of galactose and rhamnose were also present. A dispersion of the material in water, after adjustment to *ca.* pH 5 with sodium hydroxide, was treated with a pectinase preparation at 37°. After 5 days, undissolved material was isolated on the centrifuge, and hydrolysed (16 hr.) with acid. The combined acid and enzyme hydrolysates were neutralised with barium carbonate, cations were removed on IR-120(H) resin, then evaporation of the solution gave a syrup (0·7 g.) consisting of hexuronic acid (mainly), acidic oligosaccharides, and xylose; glucurono-6 ⟶ 3-lactone was not detected. Fractionation on paper chromatograms, with solvent (*a*), gave xylose (25 mg.) and a fraction (0·4 g.) containing acidic mono-, di-, and tri-saccharides. The last fraction was converted into methyl ester methyl glycoside by heating it under reflux for 16 hr. in methanolic hydrogen chloride (2% w/w; 50 c.c.). Reduction of the product with potassium borohydride (130 mg.) in water (5 c.c.) for 20 hr. at 2°, followed by hydrolysis and passage through IR-120(H) and IRA-400(CO_3) resins, afforded D-galactose (190 mg.) which after crystallisation from methanol had m. p. and mixed m. p. 163—164° and $[\alpha]_D$ +80° (equil. value; *c* 1·0).

Separation and Characterisation of Neutral Monosaccharides.—The several neutral monosaccharide fractions isolated from the plum-leaf hydrolysates as above were combined (4·4 g.), and the sugars converted into their di-*O*-*iso*propylidene derivatives (4·1 g.) by the action of acetone (100 c.c.) and concentrated sulphuric acid (5 c.c.) on the powder produced by evaporating an aqueous solution of the sugars in the presence of Celite (10 g.).[4] The *O*-*iso*propylidene compounds were dissolved in 0·1N-sulphuric acid (15 c.c.), and the solution was kept at 20° for 6½ hr. Anions were then removed on IR-4B(OH) resin, and the resultant solution shaken with chloroform (equal vol.; 4 times). Hydrolysis, by 0·5N-acid for 1 hr., of the chloroform-soluble *O*-*iso*propylidene compounds afforded a mixture (1·3 g.) of mainly galactose and arabinose, with small amounts of xylose and fucose. The *O*-*iso*propylidene compounds from the aqueous layer gave, on hydrolysis as before, a mixture (1·2 g.) of glucose and xylose, together with small amounts of arabinose and rhamnose.

Portions of these mixtures were fractionated on large paper sheets, solvents (*b*) and (*c*) being used. The sugars were eluted from the appropriate parts of the chromatograms with cold water, and the solutions so obtained were passed through IR-120(H) and IRA-400(CO_3) resins before evaporation to dryness. Eventually the following monosaccharides were obtained: (i) D-Galactose. Crystallisation of the syrup (200 mg.), $[\alpha]_D$ +75° (*c* 2·0), from methanol–ethanol afforded α-D-galactose (150 mg.), m. p. and mixed m. p. 164—165°, $[\alpha]_D$ +115° (5 min.) ⟶ +81° (equil. value; *c* 1·5). (ii) D-Glucose. The syrup (300 mg.) with $[\alpha]_D$ +45° (*c* 3·0) contained a little galactose and xylose, but partly crystallised; trituration with methanol gave α-D-glucose (40 mg.), m. p. and mixed m. p. 148°, $[\alpha]_D$ +97° (10 min.) ⟶ +51° (equil. value; *c* 0·5). The remaining material was heated under reflux in methanolic hydrogen chloride (1% w/w) for 16 hr. and yielded methyl α-D-glucopyranoside (110 mg.), m. p. and mixed m. p. 165—166°, $[\alpha]_D$ +160° (*c* 1·0). (iii) L-Arabinose. The sugar (360 mg.) crystallised spontaneously, and recrystallisation from methanol–ethanol gave L-arabinose (255 mg.), m. p. and mixed m. p. 159—160°, $[\alpha]_D$ +101° (equil. value; *c* 1·8). (iv) D-Mannose. Paper-chromatographic examination of the material in the mother-liquors from the crystallisation

of arabinose indicated the presence of small amounts of mannose and fructose in addition to arabinose, the first two of these sugars not being separated from each other (fructose was detected with orcinol–trichloroacetic acid). For the isolation of mannose, the mixture was heated in N-acid for 2 hr., to destroy the hexulose. Sheet-paper chromatography [solvent (*b*)] of the product, in which fructose was not detected, yielded syrupy D-mannose (14 mg.), $[\alpha]_D$ +12° (*c* 0·7). The derived phenylhydrazone had m. p. and mixed m. p. 188° (decomp.). (v) D-Xylose. A fraction (120 mg.) in which xylose was the main component had $[\alpha]_D$ +18° (*c* 1·2). Crystallisation from methanol–acetone afforded α-D-xylose (80 mg.), m. p. and mixed m. p. 146—147°, $[\alpha]_D$ +75° (3 min.) ⟶ +18° (equil. value; *c* 1·6). The mother-liquors contained xylose and a little arabinose, the mixture having $[\alpha]_D$ +35° (*c* 1·2). (vi) L-Rhamnose. Crystallisation of a rhamnose-containing fraction (40 mg.) from ethanol–acetone gave α-L-rhamnose monohydrate (20 mg.), m. p. and mixed m. p. 94—96°, $[\alpha]_D$ +8° (equil. value; *c* 0·9). (vii) L-Fucose. The above chromatographic separations yielded a fraction (33 mg.) containing fucose and xylose (*ca.* 1 : 1). To facilitate its characterisation, more fucose was isolated later during charcoal chromatography of larger quantities of plum-leaf monosaccharides for the isolation of (A) and (B). Then crystallisation from methanol–acetone afforded α-L-fucose, m. p. and mixed m. p. 143—144°, $[\alpha]_D$ −71° (equil. value; *c* 3·3).

Isolation of Compounds (*A*) *and* (*B*) (with J. D. ANDERSON).—Fractionation of the monosaccharide mixture from 37 g. of alcohol-insoluble plum leaf residue yielded insufficient of (A) and (B) for their characterisation. Accordingly further preparations were carried out, the following being typical: Alcohol-insoluble plum-leaf residue (350 g.) was heated with acid (5 l.), the resultant solution was brought to pH 7 with sodium hydroxide, and the bulk of the sodium sulphate removed by concentration of the solution and addition of methanol. Enrichment of (A) and (B) was effected by addition of acetone to a concentrate of the methanolic liquors, the ensuing precipitate being discarded. The product (12·5 g.) was fractionated on a squat charcoal column,[5] previously washed with water, by stepwise elution with water followed by water–ethanol mixtures. Water containing 1% of ethanol eluted a mixture (1·2 g.) of galactose, arabinose, xylose, rhamnose, and fucose; 2·5% ethanol eluted a fraction (305 mg.) which contained most of the compound (A) and some fucose; 5% ethanol eluted a fraction (120 mg.) containing (B). All of the fractions were contaminated with other substances; in those fractions containing (A) and (B) non-reducing material was also present which on hydrolysis (3 hr.) gave galactose, glucose, and arabinose in small amounts.

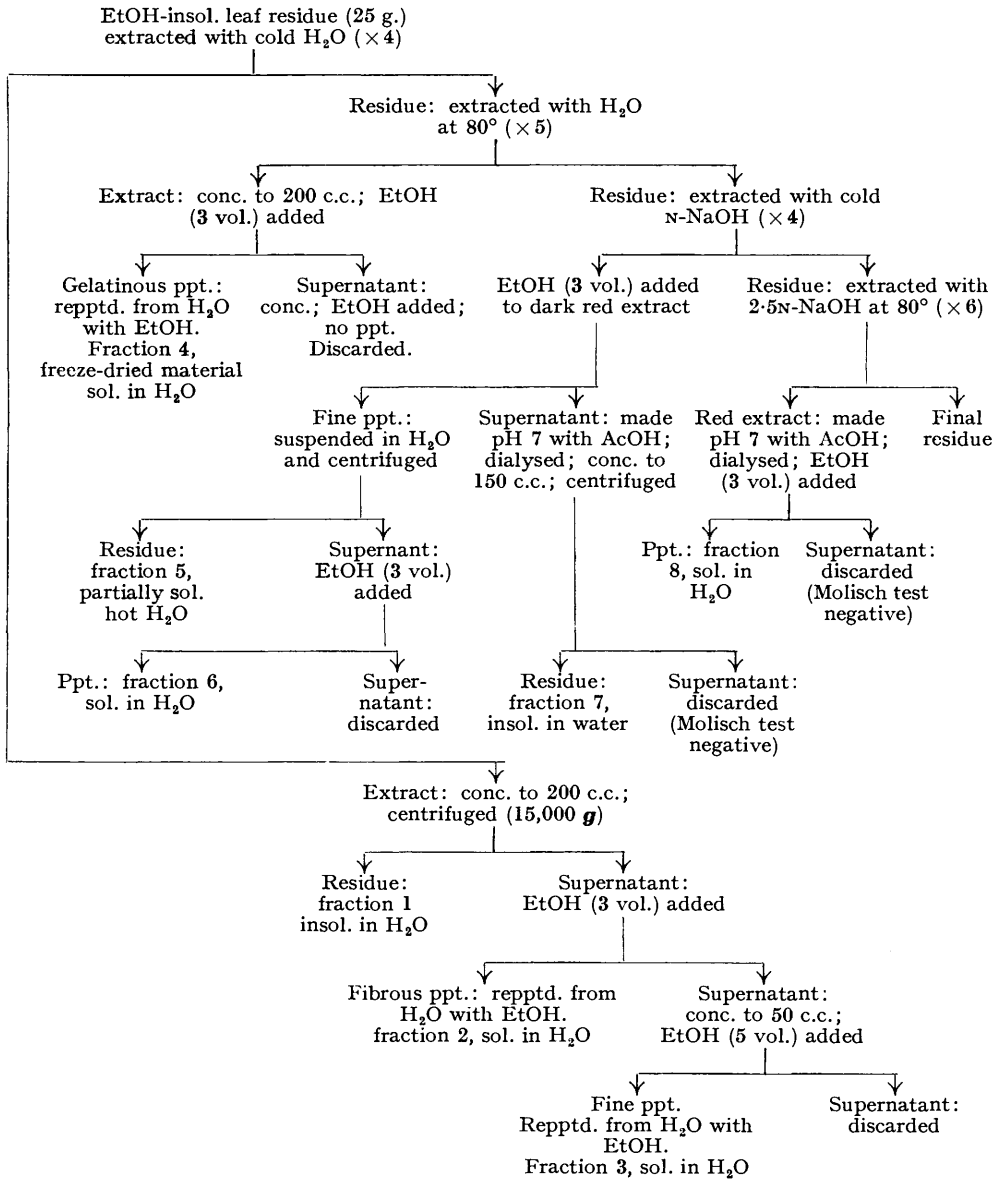
Characterisation of Compound (*A*) *as* 2-O-*Methyl*-D-*xylose*.—For the purification of (A), the material in appropriate fractions of charcoal-column effluent was heated with acid (3 hr.) which was then removed on IR-4B(OH) resin, and the products were submitted to chromatography on paper sheets with solvent (*c*). The compound was then eluted from the paper with cold water, and the solution passed through IR-120(H) and IRA-400(CO_3) resins and evaporated. The resultant colourless syrup (yield, *ca.* 20 mg. per 100 g. of dry alcohol-insoluble leaf residue) gave crystalline (A), which after recrystallisation from acetone had m. p. 132—133° (Kofler block), $[\alpha]_D$ +37° ± 6° (equil. value; *c* 0·3), R_{Rh} 1·33, 1·25, and 1·22 in solvents (*a*), (*b*), and (*c*) respectively, thus resembling a mono-*O*-methylpentose (Found: OMe, 17·9. Calc. for $C_5H_9O_4$·OMe: OMe, 18·9%). De-*O*-methylation[13] of the compound (2 mg.) was achieved by treatment with hydrobromic acid (48% w/v) at 100° for 4 min. Paper-chromatography of the neutralised (Ag_2CO_3) products indicated the presence of xylose. Quantitative estimation[14] of the formaldehyde liberated on oxidation of the compound (1·00 mg.) with sodium metaperiodate in the presence of sodium hydrogen carbonate gave 90, 148, and 184 μg. after 1·5, 3·3, and 24 hr. respectively, corresponding to yields of 0·49, 0·81, and 1·00 mole formaldehyde per mole of mono-*O*-methylpentose. The compound was indistinguishable on the paper chromatogram from 2-*O*-methylxylose, which gave a red colour with *p*-anisidine hydrochloride, but distinguishable from the 3-*O*-methyl derivative, which gave a red-brown colour and had R_{Rh} 1·38, 1·30, and 1·25 in (*a*), (*b*), and (*c*) respectively. Characterisation of (A) as 2-*O*-methyl-D-xylose was confirmed by a mixed m. p. determination (133—135°) with an authentic sample, and a comparison of their *X*-ray powder photographs. Specimens of 2- and 3-*O*-methyl-D-xylose were kindly supplied by Dr. G. O. Aspinall.

Characterisation of Compound (*B*) *as* 2-O-*Methyl*-L-*fucose*.—A fraction eluted from charcoal with 5% ethanol partly crystallised, and trituration with acetone afforded crystalline (B) (5 mg.). Paper chromatography of the rest of this fraction, and of similar fractions, gave products from

[14] Hough, Powell, and Woods, *J.*, 1956, 4799.

which further small amounts of crystalline (B) were obtained only with difficulty; crystallisation appeared to be hindered by material which was non-reducing and not held on ion-exchange resins. The crystalline sugar, the yield of which was roughly 5 mg. per 100 g. of dry alcohol-insoluble leaf residue, had m. p. 149—150°, $[\alpha]_D$ −75° ± 4° (equil. value; *c* 0·5) and R_{Rh} 1·46, 1·31, and 1·38 in (*a*), (*b*), and (*c*) respectively, suggestive of a deoxymono-*O*-methylhexose (Found OMe, 17·5. Calc. for $C_6H_{11}O_4$·OMe: OMe, 17·4%). De-*O*-methylation [13] with hydrobromic acid

Fractional extraction of plum-leaf polysaccharides.



(48% w/w) for 9 min. at 100°, followed by paper chromatography, indicated that fucose was the parent sugar. Comparison of (B) on paper chromatograms with authentic samples of 2- and 3-*O*-methyl-L-fucose [the latter having R_{Rh} 1·29 and 1·13 in solvents (*a*) and (*b*) respectively], kindly supplied by Dr. Elizabeth Percival, suggested identity with the former. Further

evidence for location of the *O*-methyl group at position 2 was obtained by periodate oxidation of the sugar (0·9 mg. samples) at pH 5·0 both before and after reduction *in situ* with sodium borohydride, and Warburg estimation of the carbon dioxide liberated (Found: 1·08 and 0·20 mol. respectively). 2-*O*-Methyl-L-rhamnose gave similar results (Found: 1·10 and 0·20 mol. respectively).[15] Identity of (B) as 2-*O*-methyl-L-fucose was confirmed by its m. p. (149—150°) when admixed with an authentic sample (m. p. 149—150°), and the identity of *X*-ray powder photographs.

Fractional Extraction of Plum-leaf Polysaccharides.—The procedure is detailed in the flow diagram. Eight fractions were extracted from alcohol-insoluble plum-leaf residue (25 g.). Nos. 1, 3, and 5—8 were brown, nos. 2 and 4 were white. After being washed with ethanol and ether, and dried under reduced pressure over phosphoric oxide, they had the properties listed in the Table.

The final residue (see flow diagram), a brown fibrous material, was washed with water until free from alkali. Hydrolysis of a small portion gave glucose and xylose (3 : 1), with a little arabinose, but most of the material remained undissolved. After two extractions with a solution of sodium hypochlorite (*ca.* 2 g.) in water (100 c.c.), containing acetic acid to bring the pH to 4, a white product was obtained; no carbohydrate was detected (Molisch test) in the extracts. Extraction of the product with 2N-sodium hydroxide gave two fractions (9*a* and *b*). Fraction (9*a*) was soluble in 2N-alkali, but insoluble in water after precipitation with ethanol. It was freed from alkali by washing it with aqueous alcohol, and dried (0·09 g.). The material was stained blue by iodine solution. After being heated with N-acid for 8 hr., it was apparently unchanged, but glucose and a trace of xylose were present in the solution. Hydrolysis with 72% w/w sulphuric acid (7 days at 20°),[16] in which the material was readily soluble, followed by N-acid at 100° for 16 hr., afforded glucose. Fraction (9*b*), insoluble in 2N-alkali, was washed with water and dried (0·13 g.). The material did not give a blue colour with iodine solution. No sugars were liberated when a portion was heated with N-acid for 8 hr., but treatment with 72% w/w sulphuric acid gave glucose.

Cellulose Estimation.[11]—The alcohol-insoluble leaf residue (0·75 g.) was heated under reflux with 80% v/v acetic acid (15 c.c.) and concentrated nitric acid (1·5 c.c.) for 20 min. After dilution of the mixture with water (50 c.c.), the insoluble cellulosic material was isolated on the centrifuge, washed with water, ethanol, and ether, and air-dried (yield, 74 mg.) (Found: N, 1·2; sulphated ash, 3·4; H_2O, 5·2%). This yield after correction for ash, moisture, and protein content, corresponds to *ca.* 8·5% of cellulose in the leaf residue. A portion of the

Fractionation of plum-leaf polysaccharides.

Fraction no.	Extraction solvent	Weight (g.)	Sulphated ash (%)	N (%)	Monosaccharides detected in hydrolysates [a]						
					Gal	Glu [b]	Arab	Xyl	Rhamn	Gal'uronic-A	2-*O*-Me-Xyl
1	Cold water	0·22	27·4	5·8	t	—	t	—	—	—	—
2	,,	0·17	22·7	Nil	+++	—	++	—	+	+	t
3	,,	1·22	81·0		—	—	—	—	—	—	—
4	Hot water	0·57	5·5	0·2	++	+	+++	+	t	+	t
5	Cold N-NaOH	0·20	35·2	2·9	—	+++	t	+	—	t	—
6	,,	3·61	56·4	3·0	+	+++	++	++	t	t	—
7	,,	0·63	4·5	12·2	—	—	—	—	—	—	—
8	Hot 2·5N-NaOH	0·34	12·3	0·6	+	++	+++	+++	+	+	t

[a] Each fraction was hydrolysed for 8 hr. and the hydrolysates neutralised with barium carbonate; +++, ++, +, t (trace), and — (absent) indicate relative amounts of the monosaccharides as detected by paper chromatography. [b] All the fractions containing glucose gave a blue colour with iodine solution.

cellulosic material (37 mg.) was treated with 72% w/w sulphuric acid (0·5 c.c.) as above.[16] After the hydrolysis, insoluble material (8 mg.) was filtered off, and the solution neutralised with barium carbonate and concentrated to a small volume. Paper chromatography indicated that glucose was the only sugar present apart from a trace of xylose, and the optical rotation

[15] Hough and Woods, *Chem. and Ind.*, 1957, 1421.
[16] Monier-Williams, *J.*, 1921, **119**, 803.

of the solution was equivalent to that of a similar solution of D-glucose (20 mg.). On this basis the cellulosic material was calculated to contain at least 49% of cellulose which corresponds to 4·8% of cellulose in the leaf residue.

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