276. *The Constitution of a Pear Cell-wall Xylan.*

By S. K. Chanda, E. L. Hirst, and E. G. V. Percival.

A xylan from the cell-wall of ripe "Conference" pears has been investigated. The polysaccharide was devoid of arabinose residues. Methylation and hydrolysis gave trimethyl D-xylopyranose (*ca.* 2%) corresponding to the presence of one non-reducing end group in 55—60 xylose units. In addition to 2 : 3-dimethyl xylose (91%), 2-methyl xylose (*ca.* 2%) and a tetramethyl aldobiuronic acid (*ca.* 2%) were also isolated. To this last product is assigned the constitution 2-methyl 3-[2 : 3 : 4-trimethyl α-D-glucuronosido]-D-xylose.

A branched structure is proposed for the xylan which appears to have 115 (±5) D-xylopyranose units in a chain with a single branch (cf. esparto xylan, *J.*, 1950, 1289) but carrying in addition a terminal D-glucopyruronic acid residue at one point. A determination of the molecular weight of the methylated xylan by the osmotic-pressure method and other observations agree with the above interpretation.

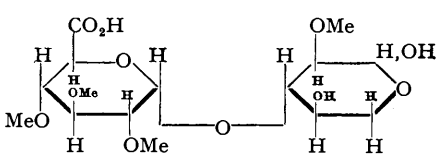
As a result of our recent study of esparto xylan (Chanda, Hirst, Jones, and Percival, *J.*, 1950, 1289), it was concluded that this polysaccharide is constructed exclusively of D-xylopyranose units, about eighty of which, linked through the 1 : 4-positions, are disposed in a singly-branched structure with the bifurcation involving the hydroxyl group on $C_{(3)}$ of one of the xylopyranose residues. The molecule thus contained one reducing and two non-reducing terminal groups.

While this work was proceeding, Dr. F. A. Isherwood and Dr. M. A. Jermyn of the Low Temperature Research Station, Cambridge, isolated from the cell-wall material of ripe pears another type of xylan devoid of arabinose residues, and kindly placed a quantity of this material at our disposal for structural investigations.

The xylan, $[\alpha]_D^{15}$ −86° (*c*, 0·6 in 0·5N-sodium hydroxide), on hydrolysis and estimation by filter-paper chromatography gave xylose in 97% yield. The presence of a uronic acid in the hydrolysate could not be conclusively determined; chromatographic separation on a heavily spotted paper, however, gave a very faint spot which corresponded possibly with glucuronic acid but could not be confirmed directly by the naphtharesorcinol test on the hydrolysate.

Methylation and fractionation of the product gave a methylated derivative {$[\alpha]_D^{12}$ −71° (in chloroform); yield 90%}. The methylated polysaccharide was hydrolysed with methanolic hydrogen chloride giving the glycosides, and from them the free sugars were obtained by hydrolysis with dilute hydrochloric acid. The hydrolysed sugars were separated on the cellulose column and the following fractions obtained: (*a*) trimethyl pentose (*ca.* 2%); (*b*) 2 : 3-dimethyl xylose (*ca.* 91%); and (*c*) 2-methyl xylose (*ca.* 2·3%) and an aldobiuronic acid (*ca.* 2·3%). The trimethyl pentose and the monomethyl xylose were both crystalline, and were identified as 2 : 3 : 4-trimethyl D-xylose and 2-methyl D-xylose respectively, confirmation being obtained by forming the anilides. The 2 : 3-dimethyl D-xylose was identified as its anilide, and as the lactone, amide, and *p*-bromophenylhydrazide of the corresponding 2 : 3-dimethyl xylonic acid.

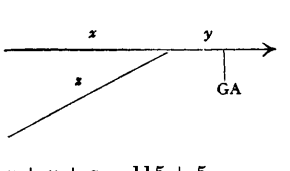
The aldobiuronic acid was identified as 2-methyl 3-(2 : 3 : 4-trimethyl α-D-glucuronosido)-D-xylose in the following way. Examination on the paper chromatogram showed the heart-shaped pattern characteristic of uronic acids (Partridge, *Biochem. J.*, 1948, **42**, 238; Reid, *J. Sci. Food Agric.*, 1950, **1**, 234). Treatment with hydrobromic acid (Hough, Jones, and Wadman, *J.*, 1950, 1702) yielded xylose and 2-methyl xylose. Reduction with lithium aluminium hydride (Lythgoe and Trippett, *J.*, 1950, 1983) followed by hydrolysis and examination on the paper chromatogram gave 2-methyl xylose and 2 : 3 : 4-trimethyl glucose in practically equimolar proportion. The equivalent weight of the methylated aldobiuronic acid (395) was, in view of its hygroscopic nature, reasonably close to the theoretical value of 382, and the high rotation ($[\alpha]_D^{20}$ +108·5°) strongly suggested an α-glycosidic linkage:



Such a compound would require one equivalent of periodate for oxidation and should give rise to a molecule of formaldehyde but no formic acid. In practice, 0·7 mole of periodate was utilised

in 24 hours and formaldehyde, but no formic acid, was produced. The alternative possibility of a linkage to $C_{(4)}$ of the xylose residue is, therefore, discounted since no attack by periodate could occur in that case.

The paper-chromatographic estimation of the different sugars obtained on hydrolysis by hypoiodite oxidation showed the following molar percentage composition : 2 : 3 : 4-trimethyl xylose (1·8—1·9), 2 : 3-dimethyl xylose (96·2—96·5) and 2-methyl xylose (1·6—1·8). A chain of 55 xylose units with one non-reducing terminal group would give trimethyl xylopyranose amounting to 1·95% of the free sugars produced on hydrolysis. The proportion of 2-methyl xylose is also of the same order but it is not to be supposed that the whole of this is derived from branching points in the xylan molecule : demethylation during hydrolysis and incomplete methylation of the starting material have to be taken into account also. As reported previously (*loc. cit.*), 2 : 3-dimethyl xylose gave only 0·3% of monomethyl xylose when treated under the hydrolytic conditions employed for methylated esparto xylan, so that the contribution from the



$x + y + z = 115 \pm 5$.
⟶ = reducing group.

FIG. 1.

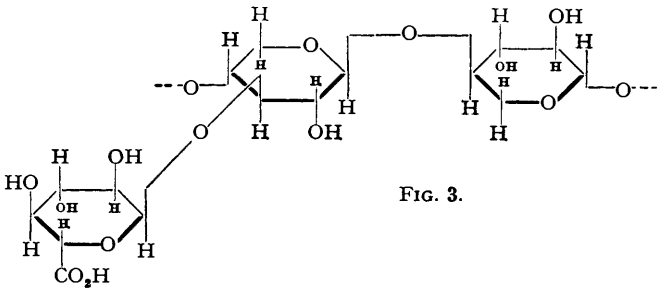
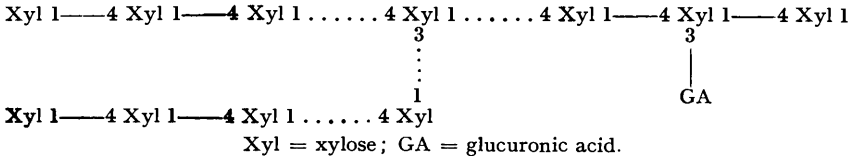


FIG. 3.



Xyl = xylose; GA = glucuronic acid.

FIG. 2.

first source must be small. It is highly probable, however, that incomplete methylation is responsible for at least half of the 2-methyl xylose detected, although it would be quite impossible to detect this by the micro-Zeisel methoxyl determination. Clearly if all the 2-methyl xylose estimated to be present in the hydrolysate had a structural significance it would be necessary to suppose the pear cell-wall xylan molecule to contain nearly as many branch points as non-reducing terminal groups, and the molecule would, therefore, contain several unit chains. There is, however, independent evidence for the conclusion that only two chains of *ca.* 55 xylose units make up the structure of the pear cell-wall xylan. The most definite is the result of the determination of the molecular weight by osmotic-pressure method (by courtesy of Mr. G. Forsyth of this Department through the co-operation of Professor H. W. Melville, F.R.S., at Birmingham University) which gave a value of 17,500 (degree of polymerisation *ca.* 110). On the basis of the above results the xylan molecule will consist of one reducing and two non-reducing terminal positions. The occurrence of an aldobiuronic acid constructed from 2-methyl xylose and trimethyl glucuronic acid also indicated that the latter is linked as a side chain in the molecule through $C_{(3)}$ of a xylose residue. One of the possible representations is given in Figs. 1 and 2, and the linkage of the D-glucuronic acid residue to the chain of D-xylopyranose units is shown in Fig. 3.

It may be recalled that a similar type of structure was proposed for esparto xylan (Chanda *et al.*, *loc. cit.*) although the maximum number of xylose residues in the molecule was estimated to be 80 in that case and no uronic acid was attached to the chain.

It should be noted that the glucuronic acid residue may be situated on any of the xylose units except the non-reducing terminal groups and the branching point.

In the present instance the type of structure proposed receives support from the estimate of the reducing power determined by the reduction of 3 : 5-dinitrosalicylic acid in alkaline solution (Meyer *et al.*, *Helv. Chim. Acta*, 1948, **31**, 103), which, applied as described previously (Chanda *et al.*, *loc. cit.*), indicated the presence of one reducing group in 110—115 units in pear

cell-wall xylan compared with one in 70—80 units in esparto xylan. On the other hand the estimation of reducing power by the hypoiodite oxidation method gave a result corresponding to 42 residues per reducing group, but a similar estimation also gave an anomalous result with esparto xylan.

The interpretation of the results of periodate oxidation is not as straightforward as in the case of the xylan from esparto grass. Pear cell-wall xylan was completely oxidised by sodium metaperiodate, consuming 1·02 mols. per $C_5H_8O_4$ residue, but it required 144 hours to oxidise the polysaccharide, as against 48 hours in the case of xylan from esparto grass. The liberation of formic acid on oxidation with potassium metaperiodate also took a different course. Even after 366 hours formic acid was still gradually accumulating, whereas the esparto xylan was completely oxidised in less than 200 hours and β-methylxylopyranoside required only 150 hours. Although the continued slow release of formic acid is a characteristic of compounds containing uronic acid groups it makes impossible a precise estimate of the molecular size by the periodate method. Nevertheless, a value taken within the range of 150—200 hours, or that given by the projection of the tangents of the curve during this period, indicated that 1 mole of formic acid was released from 18—20 $C_5H_8O_4$ units. On the type of structure proposed this corresponds to a molecule containing 90—100 pentose residues, since 2 mols. of formic acid would be released from the reducing end group and 1 mol. each from the two non-reducing xylose end groups and the glucuronic acid. As would be expected from the branched structure, after the hydrolysis of pear cell-wall xylan which had been oxidised for *ca.* 150 hours with sodium periodate a very faint trace of xylose was detected.

A comparison of the molecular weight, calculated from the specific viscosity, with that of methylated esparto xylan shows a value [41—42 xylose residues by use of the constant $K_m = 12 \times 10^{-4}$ formerly employed for cellulose derivatives by Staudinger and Reinecke (*Annalen*, 1938, **535**, 47)] of about half that obtained from esparto grass, whereas the specific viscosity of pear xylan ($\eta_{sp.}/c = 11·34$) in N-sodium hydroxide is slightly higher than that of esparto xylan ($\eta_{sp.}/c = 9·8$). Although the interpretation of viscosity measurements is open to doubt, this tends to support the branched formula for pear cell-wall xylan, as it indicated a further divergence from the ideal thread molecules.

One of the special features of interest in the xylan now discussed is the presence of glucuronic acid as a structural component. It is true that glucuronic acid appears together with xylose in such plant gums as almond-tree gum (Brown, Hirst, and Jones, *J.*, 1948, 1677), peach gum (Jones, *J.*, 1950, 534), and many others (Hirst, *J.*, 1949, 522), but in all these cases other sugar building units such as galactose, arabinose, and rhamnose are present also. Examples of polysaccharides containing xylose and glucuronic acid residues as the sole building components, a point of importance in connection with the suggestion that pentoses may originate by the decarboxylation of the corresponding hexuronic acids, are much fewer in number. One may recall the polysaccharide from *Kadzura japonica* (Nishida, Hashima, and Fukamizu, *J. Agric. Chem. Soc. Japan*, 1934, **10**, 1001; 1935, **11**, 261) and the hemicelluloses of the English oak (O'Dwyer, *Biochem. J.*, 1939, **33**, 713; 1940, **34**, 149) and the cottonwood, *Populus macdougali* (Anderson *et al.*, *J. Biol. Chem.*, 1942, **144**, 767), although in these cases a methoxyglucuronic acid is involved. The example about which most is known from the structural viewpoint is the polyuronide hemicellulose of New Zealand flax (*Phormium tenax*) (McIlroy, Holmes, and Mauger, *J.*, 1945, 796; McIlroy, *J.*, 1949, 121). In this polysaccharide the glucuronic acid residue was shown to occupy the position of a non-reducing end group as in our xylan, although the method of attachment to a relatively short main chain of xylopyranose residues linked through $C_{(1)}$ and $C_{(4)}$ appears to involve the intervention of another xylopyranose unit. It is clear that further investigations will be of great interest in view of the present deficiencies in our knowledge of the group of xylose–glucuronic acid polysaccharides.

Experimental.

The polysaccharide was prepared, and kindly made available to us, by Dr. F. A. Isherwood and Dr. M. A. Jermyn of the Low Temperature Research Station, Cambridge. The method of extraction carried out may briefly be described: Ripe "Conference" pears were peeled, cored, and frozen at −20°. The product was ground and added to sufficient boiling alcohol to give a final concentration of 90%. After an hour's boiling under reflux the residual material was filtered off and extracted two or three times with boiling 95% alcohol to remove the last traces of free sugars. The material was then broken up, and dried in the air to a powder, and finally at 100° to constant weight. The yield was 2·4% on the dressed weight of pears. The cell-wall material contained large amounts of water-soluble "pectin." It was freed from pectin by boiling it with water (4 l./100 g.). A twelve hours' extraction, followed by 3—4 periods of four hour's extraction, was usually sufficient for this purpose. The material was dried

as before (yield, 70·3%). The holocellulose was prepared from the depectinised cell-wall material (lignin 23·1, and ash 0·9%) by treatment with acidified (pH 4—5) sodium chlorite solution at 70—80° (Wise *et al.*, *Paper Trade J.*, 1946, **126**, 35). The xylan was extracted from the holocellulose by 24% potassium hydroxide solution and was precipitated by potassium Benedict solution (*i.e.* Benedict solution made with potassium instead of sodium salts). The copper complex thus formed was decomposed by glacial acetic acid, washed with 80% acetic acid to free it from copper followed by acetone, to remove the acid, and ether (yield, 25·9% on the carbohydrate content of the original holocellulose). The crude xylan (30 g.; xylose 90, glucose 7, galactose 3%, and a trace of arabinose estimated on a paper chromatogram) was purified by precipitation twice as the copper complex and regeneration of the xylan as just described. The pure polysaccharide (24·1 g.) had a low ash (0·4%) and copper content (0·018%).

Separation of the Water-soluble Fraction.—The xylan (12 g.) was treated with water (200 c.c.), with shaking, for 24 hours to extract any water-soluble fraction. The solution was centrifuged and the insoluble fraction dried by washing it with alcohol and ether and finally over phosphoric oxide in a vacuum-desiccator (98·5%). The aqueous solution was slightly turbid, and gave a precipitate with excess of alcohol (1·5% yield). The insoluble polysaccharide had $[\alpha]_D^{15}$ −86° (*c*, 0·6 in 0·5N-sodium hydroxide).

Identification and Estimation of Xylan Hydrolysate.—Filter-paper chromatographic examination (Partridge, *loc. cit.*) of the xylan hydrolysate indicated the presence of xylose—a developed paper showed a brown spot with ammoniacal silver nitrate, a pink spot with aniline oxalate, and a blue spot with naphtharesorcinol in dilute hydrochloric acid. Glucuronic acid was also detected as a faint spot in a heavily spotted paper, but could not be confirmed by naphtharesorcinol test on the hydrolysate. Chromatographic estimation of the xylose obtained on hydrolysis with 0·5N-sulphuric acid by both Somogyi's copper reagent (Flood, Hirst, and Jones, *J.*, 1948, 1679) and hypoiodite oxidation (Chanda, Hirst, Jones, and Percival, *loc. cit.*) accounted for 96—97% of xylan.

Methylation.—The xylan (9 g.) was methylated in a nitrogen atmosphere by two series of five methylations, each with methyl sulphate and sodium hydroxide (Chanda *et al.*, *loc. cit.*). The methylated polysaccharide (8·5 g.) had $[\alpha]_D^{12}$ −72° in chloroform (*c*, 1) and OMe, 38·4% (Calc. for $C_7H_{12}O_4$: OMe, 38·75%).

Methylation by methyl iodide and silver oxide. The polysaccharide was almost completely methylated by sodium hydroxide and methyl sulphate. To see whether the methoxyl content could be further increased, the above methylated xylan (8·5 g.) was dissolved in methyl iodide (200 g.) in the presence of methanol (10 c.c.) and boiled gently in the presence of silver oxide (100 g.) for 24 hours. The solution was then filtered and the oxide was repeatedly washed with hot chloroform. The filtrate on concentration gave a yellowish solid (8·4 g.). It was purified by dissolution in chloroform and precipitation with excess of dry ether. The colourless precipitate, filtered off and dried (97%), had $[\alpha]_D^{14}$ −71·5° in chloroform (*c*, 1) and OMe, 38·9%. The filtrate on concentration gave a little brown syrup (3%).

Fractionation of Methylated Xylan.—The methylated xylan (7 g.) was fractionated by various mixtures of chloroform and purified light petroleum (b. p. 65—66°) (Chanda *et al.*, *loc. cit.*). As in the case of xylan from esparto grass, with increasing concentration of chloroform in petroleum the removal of a syrup (acetone condensation product) is effected, leaving a highly purified methylated polysaccharide. The syrupy fraction had been removed during purification and also the preliminary investigation showed that very little was soluble in a solvent mixture having 15% or less of chloroform. The methylated polysaccharide started swelling in 20 : 80 chloroform–petroleum, and the polysaccharide was completely soluble in 30 : 70 chloroform–petroleum. The results are collected in the following Table.

Fraction.	Solvent, chloroform–light petroleum.	Yield, %.	Sulphated ash, %.	OMe, %.	$[\alpha]_D^{12}$.
1	20 : 80	2·3	0·12	38·6	—
2	25 : 75	6·9	0·14	38·5	−67°
3 *	30 : 70	90·5	0·19	38·7	−71°

* Fraction 3, Found : C, 52·2; H, 7·1. Calc. for $C_7H_{12}O_4$; C, 52·5; H, 7·5%.

Hydrolysis of Methylated Xylan and Separation of Methylated Xyloses.—(a) *Paper partition chromatography.* The polysaccharide (100 mg.) was hydrolysed successively with methanolic hydrogen chloride (5 c.c.; 1%) and aqueous hydrochloric acid (10 c.c.; 0·5N.) (Chanda *et al.*, *loc. cit.*), neutralised with silver carbonate, and filtered, and the silver and other basic ions were removed from the filtrate by hydrogen sulphide and "Amberlite IR100 H" ion-exchange resin. The clear solution was concentrated to a thin syrup at 35°/15 mm. Examination on a paper chromatogram with butanol–ethanol–water (4 : 1 : 5) showed, on development with aniline oxalate and ammoniacal silver nitrate, three distinct spots corresponding to a trimethyl pentose (R_G, 0·94—0·95), 2 : 3-dimethyl xylose (R_G, 0·75—0·76) and a monomethyl xylose (R_G, 0·38—0·40). A fourth sugar travelling just ahead of the starting line in the above solvent, but as far as midway between 2-methyl xylose and 2 : 3-dimethyl xylose in butanol–acetic acid–water (4 : 1 : 5), was also identified. The aniline oxalate spray showed it as a cherry-coloured heart-shaped spot characteristic of uronic acids (Partridge, *loc. cit.*; Reid, *loc. cit.*). Silver nitrate indicated a white patch on a light brown background.

The three pentoses were estimated by alkaline hypoiodite oxidation after separation on a paper chromatogram (Chanda *et al.*, *loc. cit.*) (see Table).

Sugar.	Experiment 1. Wt., mg.	Experiment 1. Molar composition, %.	Experiment 2. Wt., mg.	Experiment 2. Molar composition, %.
2 : 3 : 4-Trimethyl xylose	0·21	1·8	0·23	1·9
2 : 3-Dimethyl xylose	10·31	96·5	10·50	96·2
2-Methyl xylose	0·16	1·6	0·19	1·8

The uronic acid travelling close to the starting line did not consume any appreciable quantity of iodine. The chain length calculated on the above results indicate one non-reducing end group per 52—55 xylose units.

(b) *By the cellulose column.* Methylated xylan (5 g.) was hydrolysed on a water-bath with methanolic hydrogen chloride (500 c.c.; 1%) and the rotation observed: $[\alpha]_D^{12}$ +14° (2 hours), 36° (3 hours), 62° (4 hours), 78° (5 hours), 84° (6 hours, constant). It was observed that when the solution was kept overnight the specific rotation of the hydrolysed solution changed to +76° but, when the solution was again heated for an hour and cooled, the observed rotation corresponded to the value obtained during hydrolysis: $[\alpha]_D$ +84° ⟶ +76° in 24 hours.

The solution was neutralised with silver carbonate and filtered. The filtrate was concentrated to a syrup and dried in a vacuum-desiccator over phosphoric oxide at room temperature (6·1 g.). The syrup was rehydrolysed at 95° with hydrochloric acid (250 c.c.; 0·5N.), the rotations observed being $[\alpha]_D^{12}$ +60° (0 hour), 56·5° (1 hour), 45° (2 hours), 40° (3 hours), 36·5° (4 hours), 32·8° (5 hours), 31·5° (6 hours), 29·5° (7 hours), 29·1° (8 hours, constant). The acid solution was neutralised with silver carbonate, triturated with water, and filtered, silver ions were removed with hydrogen sulphide, and the syrup was obtained in the usual way (5·6 g.).

This syrup was separated on a column (60 × 4 cm.) of powdered cellulose (Hough, Jones, and Wadman, *J.*, 1949, 2511; Chanda, Hirst, Jones, and Percival, *loc. cit.*). The solvent employed for elution was purified light petroleum (b. p. 100—120°)–*n*-butanol (7 : 3) saturated with water. The eluate was collected in 5-c.c. batches in the automatic device which changed the tubes every 4 minutes. A fraction of the contents of each tenth tube was evaporated and the residue, if any, analysed on the paper chromatogram in chronological order. 2 : 3 : 4-Trimethyl xylose, 2 : 3-dimethyl xylose, and monomethyl xylose were completely separated from each other. Monomethyl xylose travelled slowly through the cellulose column with the above petroleum mixture, so the mobile phase was changed to *n*-butanol (100 c.c.), followed by butanol partly saturated (50% saturation) with water after the dimethyl xylose had been separated out. The tubes were then grouped to contain a pure sugar, and the solution was concentrated at 35°/15 mm. to a syrup, and dissolved in water, some waxy material separating. It may be mentioned that the formation of such waxy material could be avoided when the butanol used for separation was purified by being boiled under reflux over sodium hydroxide and distilled (unpublished). The solution was treated with a little charcoal and filtered through a well-washed bed of "Filter Cel," and the clear solution was again concentrated. The syrup was further purified by dissolving it in acetone or hot methanol when necessary, filtered, concentrated, and left to crystallise in a refrigerato (see Table).

Fraction.	Tube no.	Sugar.	Wt. (g.).
—	1—35	No sugar	—
1	36—80	Trimethyl pentose	0·1481
2	81—240	2 : 3-Dimethyl xylose	4·8120
—	241—354	No sugar	—
3	355—480	Monomethyl xylose	0·1200
—	481—600	Xylose	trace
4	Water wash	Aldobiuronic acid	0·1410

Examination of the Fractions.—Fraction 1. The syrup (148·1 mg.) did not crystallise even on long storage. Chromatographic examination showed only one spot corresponding to a trimethyl pentose. It had OMe, 46·5 (Calc. for $C_8H_{16}O_5$: OMe, 48·4%) and hypoiodite oxidation indicated 70% of pure aldopentose: $[\alpha]_D^{16}$ +25·3° in water (*c*, 1·16) in the presence of a trace of acid. A little syrup (10 mg.) was rehydrolysed with 0·5N-hydrochloric acid, and the sugar was regenerated as usual. Paper-chromatographic examination of this hydrolysed sugar showed the presence of 2 : 3-dimethyl xylose along with the original trimethyl pentose. This observation coupled with the methoxyl content and hypiodite oxidation showed that the syrup probably contained some (*ca.* 30%) 2 : 3-dimethyl methylxyloside.

The syrup (128·2 mg.) was rehydrolysed with hydrochloric acid (10 ml.; 3%) on a water bath for 5 hours. $[\alpha]_D^{16}$ +25° (0 hour), 22·6° (2 hours), 21·8° (3 hours), 21° (4 hours, constant) was observed. The solution was neutralised with silver carbonate, and the sugar obtained in the usual way (124 mg.).

Column separation of the mixture (semi-micro scale). Quantitative separation of the sugar mixture was effected in a long but narrow column (55 cm. × 1·5 cm.). The column was packed and washed as before. The syrup was soaked in at the top and the column was developed with light petroleum–butanol (7 : 3). The rate of the eluate coming out was slow but steady, and 5 c.c. were collected in 24 minutes. The chromatographic examination of the collected eluates showed that the two sugars were effectively separated. They were concentrated and purified as before. The results are summarised in the following Table.

Sugar.	Wt. (mg.).	$[\alpha]_D^{14}$.	Sugar by hypoiodite oxidation (%).	OMe (%).
Trimethyl pentose	83·2	+19·9°	97·2	48·3
2 : 3-Dimethyl xylose	35·8	+21°	92·0	33·2

The trimethyl pentose syrup crystallised completely in a day when seeded with 2 : 3 : 4-trimethyl xylose. The crystalline mass (m. p. 86°) was recrystallised from dry ether; 4 crops in almost quantitative yield were obtained, of m. p. 90° alone or on admixture with a specimen of trimethyl D-xylopyranose (Found : C, 49·2; H, 8·2. Calc. for $C_8H_{16}O_5$: C, 50·0; H, 8·3%).

Examination by the *X*-ray powder photograph method, by the kindness of Dr. C. A. Beevers, showed the substance to be trimethyl xylopyranose. The derived anilide had m. p. 103° alone or on admixture with an authentic specimen (Found : C, 63·0; H, 7·9; N, 5·2; OMe, 33·2. Calc. for $C_{14}H_{21}O_4N$: C, 62·9; H, 7·8; N, 5·2; OMe, 34·8%).

2 : 3-Dimethyl xylose (35·8 mg.) was identified by means of the anilide which gave the same m. p. (143°) as 2 : 3-dimethyl xylose anilide alone or admixed (Found : OMe, 23·7. Calc. for $C_{13}H_{19}O_4N$: OMe, 24·5%).

The amount of pure trimethyl D-xylopyranose obtained was nearly 100 ± 10 mg., corresponding to one non-reducing end group in 60 ± 5 xylose units.

Fraction 2. The syrup (4·81 g.) had $[\alpha]_D^{16}$ +22·8° (*c*, 2·95 in water), η_D^{19} 1·4760 (Found : OMe, 34·0. Calc. for $C_7H_{14}O_5$: OMe, 34·8%). Hypoiodite oxidation indicated 98—99% of aldopentose and chromatographic examination showed only 2 : 3-dimethyl xylose. The derived anilide had m. p. 145° alone or admixed with an authentic specimen and $[\alpha]_D^{15}$ +186° ⟶ +73° (45 minutes, constant; *c*, 0·9 in ethyl acetate containing 5% v/v acetic acid) (Found : C, 61·6; H, 7·5; N, 6·0; OMe, 24·1. Calc. for $C_{13}H_{19}O_4N$: C, 61·6; H, 7·6; N, 5·5; OMe, 24·5%).

Fraction 2 was converted into its furanolactone, $[\alpha]_D^{16}$ +96·5°, η_D^{16} 1·4670 (Found : OMe, 35·1. Calc. for $C_7H_{12}O_5$: OMe, 35·2%). Methanolic anhydrous ammonia quantitatively converted the lactone into the amide which crystallised on removal of the solvent. It was recrystallised from ethyl acetate and had m. p. 133° (alone or on admixture with an authentic specimen), $[\alpha]_D^{15}$ +48° in water (*c*, 0·84) (Found : C, 43·6; H, 7·8; N, 7·0; OMe, 31·6. Calc. for $C_7H_{15}O_5N$: C, 43·5; H, 7·7; N, 7·25; OMe, 32·1%).

2 : 3-Dimethyl D-xylono-*p*-bromophenylhydrazide (85% yield) had m. p. 150° (alone or on admixture with an authentic specimen) and $[\alpha]_D^{15}$ +24° (*c*, 1·22 in ethanol). No mutarotation could be observed in the presence of acid (Found : C, 43·8; H, 5·3; N, 7·8; Br, 21·6; OMe, 16·1. Calc. for $C_{13}H_{19}O_5N_2Br$: C, 43·0; H, 5·3; N, 7·7; Br, 22·0; OMe, 17·1%).

Fraction 2 was, therefore, pure 2 : 3-dimethyl D-xylose.

Fraction 3. The syrup (120 mg.) crystallised completely when seeded with 2-methyl D-xylopyranose. Chromatographic examination indicated the presence of the above sugar only. The crude crystals had $[\alpha]_D^{15}$ +28° (*c*, 1 in water), and hypoiodite oxidation indicated 96% purity. Recrystallised from methanol, it had m. p. 133° alone or on admixture with an authentic specimen (Found : C, 44·0; H, 7·6; OMe, 17·5. Calc. for $C_6H_{12}O_5$: C, 43·9; H, 7·3; OMe, 18·9%). The derived anilide had m. p. 124° alone or on admixture with an authentic specimen (Found : C, 59·6; H, 7·2; N, 5·5; OMe, 13·2. Calc. for $C_{12}H_{17}O_4N$: C, 60·25; H, 7·1; N, 5·85; OMe, 13·0%).

Fraction 4. This was a hygroscopic solid. It was purified by dissolving it (141 mg.) in hot methanol. Chromatographic examination indicated two cherry-coloured coalescing spots (R_G nearly 0·1) when a paper was irrigated with *n*-butanol–ethanol–water (4 : 1 : 5) and developed with aniline oxalate; when a similar paper was irrigated with acid solvent (*n*-butanol–acetic acid–water; 4 : 1 : 5) the spots travelled as far as 2 : 3-dimethyl xylose (distance travelled 14·3 and 16 cm. when 2 : 3-dimethyl xylose travelled 15·1 cm.). The spot having the smaller R_G value showed a heart-shaped pattern in both the solvents characteristic of uronic acid (Partridge, *loc. cit.*; Reid, *loc. cit.*), but the spots did not correspond to any methylated galacturonic acids obtained by the hydrolysis of methylated pectic acid.

Heavy spotting, however, indicated that the fraction 4 contained traces of 2-methyl xylose and xylose. It (130 mg.) was purified by passage again through a semimicro-column of cellulose (*loc. cit.*). The solvent used for the eluate was the organic fraction of the mixture butanol–acetic acid–water (4 : 1 : 5). The various fractions identified and obtained in the usual way were : (*a*) unidentified uronic acid (121·4 mg.), purified by extraction with hot methanol (110·2 mg.); (*b*) mixture of (*a*) and 2-methyl xylose (2·9 mg.); (*c*) 2-methyl xylose (1·4 mg.); and (*d*) xylose (2·1 mg.).

Demethylation of the fraction (4*a*; 10 mg.) with hydrobromic acid (Hough, Jones, and Wadman, *J.*, 1950, 1702) and examination of the demethylated product on the paper chromatogram indicated the presence of 2-methyl xylose and xylose. 2 : 3-Dimethyl xylose was also very faintly indicated but could not be confirmed.

Reduction with lithium aluminium hydride. The fraction (4*a*; 10—20 mg.) was heated in a sealed tube for 5 hours with methanolic hydrogen chloride (1%; 2 c.c.). The solution was neutralised with dry ethereal diazomethane. It was evaporated to dryness at 40°/15 mm., dissolved in dry ether (10 c.c.), and treated with an equal volume of an ethereal solution of lithium aluminium hydride (0·1 g.) (Lythgoe and Trippett, *J.*, 1950, 1983). The solution was refluxed gently for 3 hours, then cooled, and the excess of hydride was carefully destroyed with water. The solution was acidified with 2N-sulphuric acid and extracted repeatedly (10—15 times) with chloroform. The organic fraction was concentrated to a syrup at 30°/15 mm. and hydrolysed with 0·5N-hydrochloric acid (10 c.c.; 7 hours). It was neutralised with silver carbonate, and the soluble silver removed from the solution as described before. The solution was aerated and treated with " Amberlite IR100 H " to remove the basic ions, and concentrated to a thin syrup. Paper-chromatographic examination indicated the presence of 2-methyl xylose (R_G, 0·39—0·41) and 2 : 3 : 4-trimethyl glucose (R_G, 0·80—0·82; McGilvray, Ph.D. Thesis, Edinburgh, 1949)—pink and brown spots respectively with aniline oxalate. The R_G values of 2: 3 : 4-trimethyl glucose and

2 : 3 : 6-trimethyl glucose are very close, but our spot corresponded more to the former than to the latter. In any case a methoxyl group at $C_{(6)}$ is ruled out by the fact that it was the $C_{(6)}$ carboxyl group which had been converted into the primary alcohol by lithium aluminium hydride.

The amount of 2-methyl xylose and 2 : 3 : 4-trimethyl glucose was also estimated after chromatographic separation by the hypoiodite method and found to be 0·9 : 1 mol. respectively.

Periodate oxidation. The aldobiuronic acid (28 mg.), dissolved in water (10 c.c.), was converted into its sodium salt (by an excess of sodium hydroxide solution and titration of the excess of alkali with acid) and treated with sodium metaperiodate solution (0·28M.; 3 c.c.) and sodium hydrogen carbonate solution (N.; 5 c.c.). The solution was left in the dark and the amount of periodate in the solution was determined (Fleury and Lange, *J. Pharm. Chim.*, 1933, **17**, 107, 196) (Found: 24 hours, 0·7 mol./$C_{15}H_{26}O_{11}$). The oxidised solution was dextrorotatory and gave a positive test for formaldehyde with phenylhydrazine hydrochloride and potassium ferricyanide (Schryver, *Proc. Roy. Soc.*, 1910, *B*, **82**, 226).

The sodium salt of the aldobiuronic acid (21·61 mg. in 15 c.c.) was oxidised for 48 hours with sodium metaperiodate (0·25M.; 5 c.c.), the excess of periodate was destroyed with ethylene glycol (2 c.c.), and the solution titrated with alkali (0·01N.) to methyl-red (Found: 0·05 mole of formic acid per $C_{15}H_{26}O_{11}$ unit).

Fraction (**4***a*) reduced Fehling's solution and had $[\alpha]_D^{20}$ +108·5° (*c*, 2·05 in water) (Found: C, 45·7; H, 7·0; OMe, 31·8%; equiv., 395. $C_{15}H_{26}O_{11}$ requires C, 47·1; H, 6·8; OMe, 32·4%; equiv., 382).

Other Experiments.—Viscosity measurement. (1) *Xylan.* The viscosity of a 1·57% solution of the polysaccharide in N-sodium hydroxide solution was determined in an Ostwald viscometer. Mean time of flow of the solution, 693 seconds at 20°; mean time of flow of the alkali at the same temperature, 293 seconds: $\eta_{sp.}/c = 11{\cdot}34$; *c* is expressed in g.-mol. of the repeating units per titre (cf. $\eta_{sp.}/c$ of xylan from esparto grass, 9·8).

(2) *Methylated xylan* (fraction 3, cf. p. 1243). The viscosity of a 1·4% solution of the methylated xylan in *m*-cresol was similarly determined. Mean time of flow (at 20°) of the solution, 852 seconds when the mean time for the solvent was 497 seconds. $\eta_{sp.}/c = 8{\cdot}16$. $M = 6646$ from the Staudinger equation $\eta_{sp.} = K_m Mc$, where $\eta_{sp.}$ = the specific viscosity, K_m = constant, M = molecular weight, and c = concn. in g.-mol. of repeating units per litre (Staudinger and Reinecke, *Annalen*, 1938, **535**, 47; $K_m = 12 \times 10^{-4}$).

A determination of the molecular weight of the methylated xylan (3) by the osmotic-pressure method, through the kindness of Mr. G. Forsyth, gave a value of 17,500 (degree of polymerisation, *ca.* 110).

Periodate oxidation of xylan. (1) *Determination of formic acid liberated.* The method of Halsall, Hirst, and Jones (*J.*, 1947, 1399, 1427), as modified by Chanda, Hirst, Jones, and Percival (*loc. cit.*) for xylan from esparto grass, was used. The formic acid (10^{-2} moles) per $C_5H_8O_4$ units obtained was: 24 hours, 2·20; 72 hours, 3·25; 96 hours, 3·74; 144 hours, 4·51; 240 hours, 5·24; and 336 hours, 5·53. The amount of formic acid liberated was not constant even after 336 hours. A control experiment showed that β-methylxyloside was completely oxidised in 150 hours, and xylan from esparto grass in less than 200 hours.

(2) *Periodate uptake.* The xylan (100 mg.) was shaken in the dark with sodium metaperiodate solution (25 c.c.; 0·25M.), and the amount consumed was determined (Fleury and Lange, *loc. cit.*) at intervals (see Table). A faint trace of xylose could be detected in the hydrolysate of the completely oxidised material (Chanda *et al.*, *loc. cit.*).

Time (hrs.)	24	48	96	144
Mol. of periodate consumed/$C_5H_8O_4$	0·93	0·95	0·98	1·02
$[\alpha]_D$ of the oxidised solution (*c*, 0·4 as wt. of xylan)	+117°	125°	131°	136°

Reducing value of xylan. (1) Hypoiodite oxidation of the polysaccharide in the presence of carbonate–hydrogen carbonate buffer (pH 10·6) gave a value of one reducing group per 42 xylose units.

(2) *Colorimetric method.* Meyer's method (*Helv. Chim. Acta*, 1948, **31**, 103) as applied to xylan from esparto grass (Chanda, Hirst, Jones, and Percival, *loc. cit.*) was used: 62·32 mg. xylan gave log I/I_0 = 0·095 corresponding to 90 (xylose curve) or 110—115 (glucose curve) $C_5H_8O_4$ units.

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