728. *The Constitution of a Xylan from Norway Spruce* (Picea excelsa).

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Fractionation of Norway spruce hemicellulose yielded two xylan-rich components. One of these was converted into a fully methylated xylan, hydrolysis of which yielded 2 : 3 : 4-tri-*O*-methyl-, 2 : 3-di-*O*-methyl-, and 3-*O*-methyl-D-xylose, and 3-*O*-methyl-2-*O*-(2 : 3 : 4-tri-*O*-methyl-D-glucuronosyl)-D-xylose in the approximate molar ratio of 1 : 69 : 8 : 21. It is concluded from these and other experiments that this xylan has a straight chain of 80—85 1 : 4-linked β-D-xylopyranose residues with every fifth residue carrying a terminal 4-*O*-methyl-D-glucuronic acid residue linked through position 2.

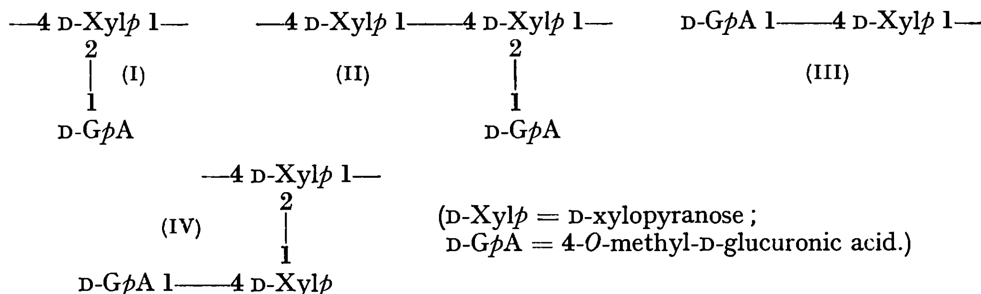
Recent investigations [1] have indicated that the xylans from land plants all contain backbones of 1 : 4-linked β-D-xylopyranose residues, but that the various xylans differ considerably in the nature, number, and mode of linkage of other sugar residues attached as side-chains. For example, beechwood xylan [2] contains side-chains of single 4-*O*-methyl-D-glucuronic acid residues linked to $C_{(2)}$ of xylose, whereas the xylans from esparto grass and the cereal straws are often characterised by the presence of L-arabofuranose residues linked to xylose through $C_{(3)}$, although in some cases D-glucuronic acid residues are also found. It was of interest, therefore, to extend investigations to the xylans from the coniferous woods or gymnosperms. The isolation of xylobiose and the aldobiouronic acid, 2-*O*-(4-*O*-methyl-D-glucuronosyl)-D-xylose, from the partial acid hydrolysis of black spruce (*Picea nigra*) and Scots pine (*Pinus sylvestris*) [3] suggested that xylans of the type found in beechwood might also be present in these woods. On the other hand, arabinose has been found in the hydrolysates from coniferous woods,[4] and no evidence was available as to whether this sugar was a constituent of a xylose-containing polysaccharide. This paper describes the structure of a xylan isolated from Norway spruce wood (*Picea excelsa*).

The spruce hemicellulose was isolated by extraction of the delignified sawdust with cold aqueous sodium hydroxide; previous experiments had shown that only small yields of alkali-soluble polysaccharides could be obtained by direct extraction of the wood without removal of lignin with chlorous acid. The crude hemicellulose yielded a complex mixture of sugars on hydrolysis, but by careful precipitation from aqueous solution and by

[1] Hirst, *J.*, 1955, 2974.
[2] Aspinall, Hirst, and Mahomed, *J.*, 1954, 1734.
[3] Gorrod and Jones, *J.*, 1954, 2522.
[4] Gustafsson, Pettersson, and Lindh, *Paperi ja Puu*, 1951, **33**, 300.

precipitation of contaminating polysaccharides as the copper complex two xylan-rich fractions were isolated. Both of these fractions gave, on hydrolysis, acidic oligosaccharides, reduction of whose methyl ester methyl glycosides with potassium borohydride, followed by hydrolysis, yielded 4-*O*-methyl-D-glucose and xylose. As 4-*O*-methyl-D-glucose was not detected on hydrolysis of the xylan fractions, it is clear that this sugar was formed by the reduction of 4-*O*-methyl-D-glucuronic acid, a fairly common constituent of plant polysaccharides.

One of the xylan fractions was converted into its fully methylated derivative, during which process the contaminating polysaccharides containing sugar residues other than xylose and glucuronic acid were lost. Hydrolysis of the methylated xylan gave 2 : 3 : 4-tri-*O*-methyl-, 2 : 3-di-*O*-methyl-, and 3-*O*-methyl-D-xylose, together with a mixture of acidic components. The acidic fraction had equivalent weight 378 (Calc. for a glucuronosylxylose tetramethyl ether, 382), but chromatography indicated the presence of at least two components, one of which travelled on the chromatogram at the same rate as 2 : 3 : 4-tri-*O*-methyl-D-glucuronic acid. The major component of the acidic fraction was identified as 3-*O*-methyl-2-*O*-(2 : 3 : 4-tri-*O*-methyl-D-glucuronosyl)-D-xylose in the following way. Reduction of the derived methyl ester methyl glycoside with lithium aluminium hydride followed by hydrolysis gave 2 : 3 : 4-tri-*O*-methylglucose and 3-*O*-methylxylose, together with a small amount of 2 : 3-di-*O*-methylxylose. A portion of the reduced acidic fraction was remethylated and after hydrolysis yielded two main components, 2 : 3 : 4 : 6-tetra-*O*-methyl-D-glucose and 3 : 4-di-*O*-methyl-D-xylose, with traces of 2 : 3 : 4-tri-*O*-methyl- and 3-*O*-methyl-xylose, and 2 : 3 : 4-tri-*O*-methylglucose; no 2 : 3-di-*O*-methylxylose could be detected. It follows from these observations that the 4-*O*-methyl-D-glucuronic acid residues are attached directly to the backbone of D-xylose residues through position 2 as in (I). The chromatographic identification of 2 : 3-di-*O*-methylxylose on hydrolysis of the reduced acidic fraction, and of 2 : 3 : 4-tri-*O*-methyl- and 3-*O*-methyl-xylose on hydrolysis after further methylation suggests that the acidic fraction also contained a trisaccharide derived from the xylan as in (II). The absence of 2 : 3-di-*O*-methylxylose on hydrolysis after remethylation excludes the possibility that the backbone of 1 : 4-linked xylose units might be terminated by a glucuronic acid unit as in (III) or that the side-chains might consist of disaccharide units as in (IV).



The hydrolysis products from the methylated xylan, namely, 2 : 3 : 4-tri-*O*-methyl-, 2 : 3-di-*O*-methyl-, and 3-*O*-methyl-D-xylose, and the acidic fraction (calculated as tetra-*O*-methylaldobiouronic acid) were isolated in the approximate molar ratio of 1 : 69 : 8 : 21. A molecular-weight determination by the isothermal-distillation method (by the courtesy of Dr. C. T. Greenwood and Mr. W. N. Broatch) gave a value of 17,000 $\pm$ 500 (degree of polymerisation, 101 $\pm$ 3) for the methylated xylan. This value, taken together with the value of one non-reducing xylose end-group per *ca.* 100 sugar residues, indicates that the backbone of xylose residues is unbranched. It is concluded, therefore, that this xylan has a straight chain of 80—85 1 : 4-linked β-D-xylopyranose residues, with, on the average, every fifth residue carrying a terminal 4-*O*-methyl-D-glucuronic acid residue linked through $C_{(2)}$. This xylan is probably only one of many closely related molecular species present in the wood, and it is of interest that no arabinose residues were found. It is possible,

therefore, that the arabinose residues present in Norway sprucewood may occur in association with galactose residues, as is the case in the ε-galactan from European [5] and North American [6] larches, and in the arabogalactan from Jeffery pine.[7] However, on present evidence the presence, in spruce, of araboxylans, of the type found in the cereal straws, cannot be excluded.

These results indicate that this Norway spruce xylan resembles closely the hemicellulose A from beechwood,[2] although differing slightly in chain length and in the proportion of 4-*O*-methyl-D-glucuronic acid residues linked as side-chains. Although no detailed structural studies have been carried out on the xylose-containing polysaccharides from aspen,[8] black spruce, and Scots pine [3] woods, it will be recalled that evidence for the presence of xylans of the same general type has been obtained by the isolation of xylobiose and the aldobiouronic acid, 2-*O*-(4-*O*-methyl-D-glucuronosyl)-D-xylose, on partial acid hydrolysis of these woods. Whilst the xylans from different plants [1] exhibit considerable variations in detailed molecular structure, our present knowledge suggests that the xylans from both deciduous and coniferous woods contain the same structural features and differ only in chain length and/or number of uronic acid residues attached as side-chains. However, further investigations of other wood xylans will be necessary before such a generalisation could be regarded as established.

Experimental

Paper chromatography was carried out on Whatman No. 1 filter paper, with the upper layers of the following solvent systems (v/v): (A) butan-1-ol–benzene–pyridine–water (5 : 1 : 3 : 3); (B) butan-1-ol–ethanol–water (5 : 1 : 4); (C) butan-1-ol–formic acid–water (500 : 115 : 385). Paper ionophoresis was carried out [9] in borate buffer at pH 10. Optical rotations were observed at room temperature (16—18°).

Extraction and Fractionation of Spruce Hemicellulose.—Extractive-free sawdust was delignified by Wise, Murphy, and D'Addieco's [10] method. In a typical extraction spruce holocellulose (40 g.) was treated with cold 4% aqueous sodium hydroxide under nitrogen for 18 hr. The filtrate was acidified with acetic acid to pH 4 and the crude hemicellulose (3 g.) was precipitated by the addition of two volumes of acetone. Chromatographic examination of the hydrolysate showed xylose, arabinose, mannose, an aldobiouronic acid, and traces of glucose and galactose. Fractionation of the hemicellulose was carried out as follows: Hemicellulose (1 g.) was dissolved in water (100 ml.), acetone (67 ml.) was added, and the solution was acidified to pH 3—4 by sulphuric acid. The resulting precipitate was discarded, sodium carbonate solution was added to bring the supernatant liquor to pH 5—6, and acetone was added until further polysaccharide was precipitated. The precipitated polysaccharide (xylan A; 0·2 g.) was separated and dried.

Spruce holocellulose, exhaustively extracted with 4% aqueous sodium hydroxide, was further extracted with cold 10% aqueous sodium hydroxide under nitrogen for 18 hr., the extract was neutralised with acetic acid, and the precipitate (mainly glucan and mannan) was discarded. Addition of acetone (0·5 vol.) to the supernatant liquor yielded a further precipitate, which gave on hydrolysis xylose, mannose, glucose, an aldobiouronic acid, and a trace of galactose. A xylan-rich fraction was obtained by dissolving the crude hemicellulose (12 g.) in 4% sodium hydroxide solution (1 l.) and adding to the solution glycerol (3 ml.) followed by 0·5M-copper sulphate solution. The pale blue precipitate, which was first formed, was removed at the centrifuge and the addition of copper sulphate was stopped before the dark blue gelatinous precipitate of the xylan complex appeared. The unprecipitated polysaccharide was recovered by neutralisation of the solution with acetic acid followed by precipitation with acetone (*ca.* 0·5 vol.). This procedure was repeated four times until the addition of copper sulphate no longer resulted in the formation of a pale blue precipitate. The polysaccharide (xylan B; 3 g.)

[5] Campbell, Hirst, and Jones, *J.*, 1948, 774; Jones, *J.*, 1953, 1672; Aspinall, Hirst, and Ramstad, unpublished results.
[6] White, *J. Amer. Chem. Soc.*, 1941, **63**, 2871; 1942, **64**, 302, 1507, 2838.
[7] Wadman, Anderson, and Hassid, *ibid.*, 1954, **76**, 4097.
[8] Jones and Wise, *J.*, 1952, 3389.
[9] Consden and Stainer, *Nature*, 1952, **169**, 783.
[10] Wise, Murphy, and D'Addieco, *Paper Trade J.*, 1946, **122**, 35.

isolated from these fractionations gave on hydrolysis xylose and an aldobiouronic acid, together with smaller amounts of mannose, glucose, and galactose.

Examination of Xylan-rich Fractions.—Xylan A had $[\alpha]_D$ −38° (*c*, 0·39 in N-sodium hydroxide) [Found: uronic anhydride (by decarboxylation), 15·0; OMe, 2·7%] and chromatographic examination of the hydrolysate showed the presence of xylose (58%), arabinose (17%), and glucose + galactose (8%). Xylan B had $[\alpha]_D$ −44° (*c*, 0·32 in N-sodium hydroxide) (Found: uronic anhydride, 18·9; OMe, 1·8%) and chromatographic examination of the hydrolysate showed the presence of xylose (63%), mannose (13%), and glucose + galactose (7%).

Xylan A (3 g.) was hydrolysed with N-sulphuric acid (50 ml.) at 100° for 4 hr. The hydrolysate was neutralised with barium carbonate, and barium ions were removed with Amberlite resin IR-120. The resulting solution was poured on to a column of charcoal–Celite, and the monosaccharides were eluted with water. Elution with ethyl methyl ketone–water (1 : 19) yielded a fraction containing aldobiouronic acid, which was converted into the methyl ester methyl glycoside and treated with a solution of potassium borohydride (300 mg.) in water (20 ml.) for 24 hr. Excess of borohydride was destroyed by the addition of acetic acid, and the solution was de-ionised by successive treatments with Amberlite resins IR-120 and IR-4B. The resulting syrup was hydrolysed with 0·8N-hydrochloric acid (15 ml.) for 6 hr. at 100°. Chromatographic examination of the hydrolysate showed the presence of 4-*O*-methylglucose and xylose. The fastest-moving component (56 mg.) was separated on filter sheets, with solvent A, but attempts to characterise the sugar by conversion into the crystalline 4-*O*-methyl-D-glucosazone failed. However, an impure fraction, shown by circular paper chromatography [11] to contain mainly the desired compound, gave an *X*-ray powder photograph with the same lines as those from an authentic sample.

Xylan B (300 mg.) was hydrolysed with N-sulphuric acid (10 ml.) at 100° for 10 hr. The hydrolysate was neutralised with barium carbonate, de-ionised with Amberlite resin IR-120, and concentrated to a syrup. The acidic component (23 mg.) was separated on filter sheets with solvent A, and was converted into the methyl ester methyl glycoside, which was treated with a solution of potassium borohydride (15 mg.) in water (5 ml.) for 24 hr. After destruction of the excess of borohydride with acetic acid, 2N-hydrochloric acid (5 ml.) was added, and the solution was heated at 100° for 6 hr. After neutralisation and de-ionisation, chromatographic examination of the hydrolysate showed the presence of 4-*O*-methylglucose, xylose, and a trace of glucose.

Methylation of Xylan.—Xylan B (10 g.) was methylated fifteen times with methyl sulphate and sodium hydroxide. The partially methylated polysaccharide, suspended in acetone–water (1 : 1), was stirred with Amberlite resin IR-120 to remove sodium ions, the solution was taken to dryness, and the polysaccharide (4·7 g.) was precipitated from chloroform solution by the addition of light petroleum (b. p. 60—80°). Methylation of the product with methyl iodide and silver oxide gave methylated spruce xylan (4·0 g.) {OMe, 40·3%; $[\alpha]_D$ −43·7° (*c*, 0·25 in $CHCl_3$)}.

Hydrolysis of Methylated Xylan and Separation of Methylated Sugars.—Methylated xylan (3·0 g.) was dissolved in 2N-hydrochloric acid (230 ml.) and kept at room temperature for 17 days. After addition of water (230 ml.), the solution was heated at 100° for 4 hr. and kept at room temperature for 20 hr. This procedure was repeated three times (constant rotation), and a small quantity of insoluble material (*ca.* 0·1 g.) was separated and hydrolysed by successive treatments with boiling methanolic hydrogen chloride and with N-hydrochloric acid at 100°. The combined hydrolysates were neutralised with silver carbonate, the acidic components were converted into barium salts by treatment with barium carbonate, and the solution was concentrated to a syrup (2·9 g.).

The methylated sugars (2·9 g.) were fractionated on cellulose [11] (74 × 3·8 cm.) with light petroleum (b. p. 100—120°)–butan-1-ol (7 : 3; later, 1 : 1) saturated with water, and butan-1-ol partly saturated with water as eluants, to give five fractions, and a further fraction was obtained by elution of the cellulose with water. Apart from fraction 1 (23 mg.), all other fractions were contaminated with acidic components. Fractions 2 (88 mg.), 4 (0·212 g.), and 6 (0·564 g.) contained only acidic substances and were combined. Portions of fractions 3 (1·510 g.) and 5 (0·188 g.) were each separated on filter sheets with solvent B, and the proportions of acidic and neutral components were determined. The results showed that the following methylated sugars were present in the hydrolysate: tri-*O*-methylxylose [0·023 g., 1·03% (mol.)], di-*O*-methylxylose (1·435 g., 69·2%), mono-*O*-methylxylose (0·161 g., 8·4%), and methylated aldobiouronic acid [0·949 g., 21·3% (calc. as tetramethylaldobiouronic acid)].

[11] Hough, Jones, and Wadman, *J.*, 1949, 2511.

Examination of Neutral Sugars.—Fraction 1 crystallised and had m. p. and mixed m. p. (with 2 : 3 : 4-tri-*O*-methyl-D-xylose) 88—91° (Found: OMe, 48·0. Calc. for $C_8H_{16}O_5$: OMe, 48·4%). The neutral component from fraction 3 (Found: OMe, 34·6. Calc. for $C_7H_{14}O_5$: OMe, 34·8%) was identified as 2 : 3-di-*O*-methyl-D-xylose by conversion into the aniline derivative, m. p. and mixed m. p. 122°, and into 2 : 3-di-*O*-methyl-D-xylonamide, m. p. and mixed m. p. 132°. The neutral component from fraction 5 (Found: OMe, 17·9. Calc. for $C_6H_{12}O_5$: OMe, 18·9%) travelled on the chromatogram at the same rate as 2- and/or 3-*O*-methyl-D-xylose, but ionophoretic examination showed the presence of the 3-methyl ether with only traces of the 2-methyl ether.

Examination of the Acidic Fraction.—Chromatographic examination in solvent C of the acidic fractions showed that they all contained two components, one travelling at the same rate as 2 : 3 : 4-tri-*O*-methyl-D-glucuronic acid. The combined acidic fractions had equiv. 378 (calc. for a tetra-*O*-methylaldobiouronic acid, $C_{15}H_{26}O_{11}$, 382).

The acid (200 mg.) was refluxed with methanolic 1% hydrogen chloride (40 ml.) for 6 hr. The product, after neutralisation, was dissolved in dry ether (65 ml.), lithium aluminium hydride (300 mg.) was added, and the solution was refluxed for 6 hr. Excess of hydride was destroyed by water, and the solution was acidified with sulphuric acid and extracted with chloroform (6 × 50 ml.). The extract was evaporated to a syrup, which was hydrolysed by hot 0·8N-hydrochloric acid for 6 hr. After neutralisation with silver carbonate, part of the hydrolysate was separated on filter sheets with solvent B, to give fractions *a* (61 mg.) and *b* (16 mg.). Fraction *a* contained two components, chromatographically and ionophoretically indistinguishable from 2 : 3 : 4-tri-*O*-methyl-D-glucose (major) and 2 : 3-di-*O*-methyl-D-xylose (minor) severally; the major component was further separated and on demethylation gave only glucose (Found: OMe, 40·0. Calc. for $C_9H_{18}O_6$: OMe, 41·9%). Fraction *b* travelled on the chromatogram at the same rate as 2- and/or 3-*O*-methyl-D-xylose, but at a different rate from the 4-methyl ether. Ionophoretic examination showed that only the 3-methyl ether was present.

The acid (375 mg.) was refluxed with methanolic 1% hydrogen chloride (60 ml.) for 6 hr. After neutralisation with silver carbonate and removal of solvent, the methyl ester methyl glycoside was reduced with lithium aluminium hydride (300 mg.) in boiling methylal (70 ml.) for 2 hr. Excess of hydride was destroyed by water, the methylal layer was separated, and the aqueous layer was extracted with chloroform and ether. The aqueous layer was acidified with dilute sulphuric acid, the sulphate and aluminium ions were removed as precipitates on addition of barium hydroxide, and the barium and lithium ions were removed as the insoluble carbonates. The resulting solution was taken to dryness and combined with the organic extracts to give a syrup (241 mg.). The reduced aldobiouronic acid was remethylated with methyl iodide and silver oxide, and the methylated disaccharide (259 mg.) was hydrolysed with 0·8N-hydrochloric acid (20 ml.) at 100° for 6 hr. and after neutralisation gave a syrup (208 mg.). Chromatographic examination of the hydrolysate showed two main conponents, tetra-*O*-methylglucose and 3 : 4-di-*O*-methylxylose, together with traces of 2 : 3 : 4-tri-*O*-methyl- and 3-*O*-methyl-xylose, and 2 : 3 : 4-tri-*O*-methylglucose; no 2 : 3-di-*O*-methylxylose could be detected. The main components were separated on filter sheets with solvent B, to yield 2 : 3 : 4 : 6-tetra-*O*-methyl-D-glucose, m. p. [after recrystallisation from light petroleum (b. p. 40—60°) containing a little ether] and mixed m. p. 95·5—96·5°, and 3 : 4-di-*O*-methyl-D-xylose, identified by conversion into 3 : 4-di-*O*-methyl-D-xylonolactone, m. p. and mixed m. p. 64—66°.

The authors thank Professor E. L. Hirst, F.R.S., for his interest and advice, Messrs. Alex. Cowan and Sons Ltd. for financial assistance (to M. E. C.), and the Rockefeller Foundation for a grant.

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