- Dutton, G. J. & Storey, I. D. E. (1954). *Biochem. J.* **57**, 275.
- Fishman, W. H. & Green, S. (1957). *J. biol. Chem.* **225**, 435.
- Ginsburg, V., Weissbach, A. & Maxwell, E. S. (1958). *Biochim. biophys. Acta*, **23**, 649.
- Glaser, L. & Brown, D. H. (1955). *Proc. nat. Acad. Sci., Wash.*, **41**, 253.
- Grodsky, G. M. & Carbone, J. V. (1957). *J. biol. Chem.* **226**, 449.
- Hanes, C. S. & Isherwood, F. A. (1949). *Nature, Lond.*, **164**, 1107.
- Hartiala, K. J. V. (1955). *Ann. Med. exp. Fenn.* **33**, 239.
- Hartiala, K. J. V. (1961). *Biochem. Pharmacol.* **6**, 82.
- Ibsen, H. L. (1928). *J. exp. Zool.* **51**, 51.
- Isselbacher, K. J. (1961). *Biochem. biophys. Res. Commun.* **5**, 243.
- Karunairatnam, M. C., Kerr, L. M. H. & Levvy, G. A. (1949). *Biochem. J.* **45**, 496.
- Kent, P. W. & Pasternak, C. A. (1958). *Biochem. J.* **69**, 453.
- Kerr, L. M. H., Graham, A. F. & Levvy, G. A. (1948). *Biochem. J.* **42**, 191.
- Krebs, H. A. & Henseleit, K. (1932). *Hoppe-Seyl. Z.* **210**, 33.
- Lester, R. & Schmid, R. (1961). *Nature, Lond.*, **190**, 452.
- Levy, G. A. (1952). *Biochem. J.* **52**, 464.
- Levy, G. A., McAllan, A. & Marsh, C. A. (1958). *Biochem. J.* **69**, 22.
- Levy, G. A. & Storey, I. D. E. (1949). *Biochem. J.* **44**, 295.
- Lipschitz, W. L. & Bueding, E. (1939). *J. biol. Chem.* **129**, 333.
- Malloy, H. T. & Evelyn, K. A. (1937). *J. biol. Chem.* **119**, 481.
- Markham, R. & Smith, J. D. (1949). *Biochem. J.* **45**, 294.
- Markham, R. & Smith, J. D. (1951). *Biochem. J.* **49**, 407.
- Markowitz, A., Cifonelli, J. A. & Dorfman, A. (1959). *J. biol. Chem.* **234**, 2343.
- Marsh, C. A. & Levvy, G. A. (1958). *Biochem. J.* **68**, 610.
- Paladini, A. C. & Leloir, L. F. (1952). *Biochem. J.* **51**, 426.
- Partridge, S. M. (1949). *Symp. biochem. Soc.* **3**, 52.
- Paul, J. (1951). Ph.D. Thesis: University of Glasgow.
- Pogell, B. M. & Leloir, L. F. (1961). *J. biol. Chem.* **236**, 293.
- Potter, V. R. (1948). *Meth. med. Res.* **1**, 317.
- Schachter, D., Kass D. & Lannon, T. J. (1959). *J. biol. Chem.* **234**, 201.
- Schmid, R. (1956). *Science*, **124**, 76.
- Schneider, W. C. & Hogeboom, G. H. (1950). *J. biol. Chem.* **183**, 123.
- Shirai, Y. & Ohkubo, T. (1954). *J. Biochem., Tokyo*, **41**, 341.
- Sie, H.-G. & Fishman, W. H. (1957). *J. biol. Chem.* **225**, 453.
- Smith, H. & Gallop, R. C. (1953). *Biochem. J.* **53**, 666.
- Stevenson, I. H. & Dutton, G. J. (1960). *Biochem. J.* **77**, 19P.
- Storey, I. D. E. (1950). *Biochem. J.* **47**, 212.
- Storey, I. D. E. & Dutton, G. J. (1955). *Biochem. J.* **59**, 279.
- Strominger, J. L., Maxwell, E. S., Axelrod, J. & Kalckar, H. M. (1957). *J. biol. Chem.* **224**, 79.
- Tapley, D. F., Herz, R., Ross, J. E., Deuel, T. F. & Leventer, L. (1960). *Biochim. biophys. Acta*, **43**, 344.
- Werner, I. (1953). *Uppsala Läkfören, Forh.* **58**, 1.
- Zini, F. (1952). *Sperimentale*, **102**, 40.

Biochem. J. (1962) **82**, 340

Changes in the Chemical Composition of a Cambial Cell during its Differentiation into Xylem and Phloem Tissue in Trees

3. XYLAN, GLUCOMANNAN AND α -CELLULOSE FRACTIONS*

BY J. P. THORNBUR AND D. H. NORTHCOTE

Department of Biochemistry, University of Cambridge

(Received 10 July 1961)

Thornber & Northcote (1961b) found that, in both angiosperms and gymnosperms, the percentage of 4-O-methylglucuronic acid in the hemicellulose component of cambial cell walls was less than that in the hemicellulose synthesized during the secondary thickening of these walls. The xylns contain all the 4-O-methylglucuronic acid residues present in the cell walls of higher plants. Therefore these polysaccharides have now been isolated from the cambial, phloem and xylem cells of pine and sycamore, and their structures compared.

* Part 2: Thornber & Northcote (1961b).

The nature of the α -cellulose fraction and glucomannan components of the cell wall synthesized at different stages of development has not been extensively investigated in woody tissues. In the work described here some comparisons have been made of material prepared from cambium and differentiated tissues.

EXPERIMENTAL

Materials

Phloem, cambial and xylem material. This was obtained from pine (*Pinus sylvestris*) saplings (8 years old) grown in

Mildenhall Woods, Thetford, and from sycamore (*Acer pseudoplatanus*) saplings (5 years old) grown at Vatches Farm, Barton, Cambridge. The saplings were cut down on 16 and 17 April 1961.

The branches were removed from each sapling and the bark was peeled from the stem. Approximately 2 g. of the bark was chopped and passed through a 16-mesh sieve, and used for the preparation of the polysaccharides from the phloem. The soft cambial tissue, exposed by removal of the bark, was scraped off the wood and immediately dropped into boiling water. The cellular regions that were removed by these procedures are shown in Plate 1. The cambial tissue was identical with that used for the analyses reported by Thornber & Northcote (1961*a, b*).

Approximately 2 g. of the wood which remained after removal of the cambial tissue (Plate 1) was treated in the same manner as the phloem and used for the preparation of the polysaccharides from the xylem.

Microscopical examination of material. Samples were fixed in dilute chromic acid fixative (Johansen, 1951), dehydrated and embedded in wax. A sledge microtome was used to cut sections about 12μ thick. The sections were stained with 1% (w/v) safranin in 48% ethanol, and counter-stained with picric aniline blue (1 g. of aniline blue and 0.5 g. of picric acid in 900 ml. of 90% ethanol). The sections were mounted in Canada balsam and photo-

graphed with the camera attachment for the microscope (W. Watson and Sons Ltd., Barnet, Herts.).

Analytical methods

Ash. This was obtained from a portion of the dried polysaccharide which had been maintained at dull red heat (500–600°) in a muffle furnace for 3 hr.

Methoxyl groups. The weight of methoxyl groups in each xylan preparation was determined by the method of Pregl (1937).

Acetyl groups. The weight of acetyl groups in each xylan preparation was determined by the method of Whistler & Jeanes (1943).

Chromatography and quantitative estimation of carbohydrate components of the polysaccharide fractions. The methods were identical with those described by Thornber & Northcote (1961*b*).

Identification of starch in polysaccharide preparations. The polysaccharides were treated with a dilute solution of I_2 and examined under a microscope for a purple coloration.

Preparation of polysaccharide fractions and lignin (Fig. 1)

All tissues and polysaccharide preparations were dried at room temperature at 0.1 mm. Hg over P_2O_5 .

Fig. 1. Fractionation scheme of phloem, cambial and xylem material.

Extractive-free material. Dried material (approximately 1 g.) or fresh cambial tissue (approximately 4 g.) was extracted with water and ethanol-benzene (1:2, v/v) as described by Thorner & Northcote (1961a).

Lignin. A portion of the extractive-free material (100 mg.) was used for the estimation of lignin by the method of Ritter, Seborg & Mitchell (1932).

Pectic substances. The remainder of the extractive-free material (approx. 0.9 g.) was extracted with 0.05M-EDTA, pH 4.5, (50 ml.) at 60° for 6 hr. The pectic substances were isolated from the extract (Thorner & Northcote, 1961a), dried and weighed.

Removal of lignin. The material which had been extracted with EDTA was suspended in ice and water. Chlorine was bubbled through the suspension for 5 min. The chlorinated lignin was exhaustively extracted with ethanolic 3% (v/v) ethanolamine at 60°. This procedure was repeated three times. The dried residue (holocellulose) was washed, dried and weighed.

Xylan fraction. Dimethyl sulphoxide (50 ml.) (technical grade; L. Light and Co. Ltd., Colnbrook, Bucks.) was added to the holocellulose and the mixture was shaken at 25° for 24 hr. The extract was removed by filtration and the residue was washed with dimethyl sulphoxide (25 ml.) and water (50 ml.) (Hagglund, Lindberg & Macpherson, 1956). The extract and washings were combined and dialysed against running tap water for 8 hr. and against distilled water for a further 16 hr. Ethanol was added to the dialysed solution to give a final concentration of over 80% and the xylan fraction was precipitated. It was allowed to settle for 18 hr., centrifuged, washed with ethanol, dried and weighed. The material insoluble in dimethyl sulphoxide was washed with water, ethanol and acetone, dried and weighed.

Polysaccharides soluble in 24% (w/v) potassium hydroxide (hemicellulose fraction I). The residue from the extraction with dimethyl sulphoxide was further extracted with 24% (w/v) KOH solution (50 ml.) under N₂ at 25° for 4 hr. (Wise, Murphy & D'Addieco, 1946). The polysaccharides (hemicellulose fraction I) were isolated from the extract as described by Thorner & Northcote (1961a). The material which was insoluble in KOH was washed with ethanol, dried and weighed.

Glucomannan fraction, polysaccharides soluble in 17.5% (w/v) sodium hydroxide (hemicellulose fraction II) and α -cellulose. The insoluble residue from the treatment with KOH was extracted with 17.5% (w/v) NaOH solution (50 ml.) that contained 4% (w/v) H₃BO₃ (Jones & Painter, 1957) for 4 hr. under N₂ at 25°. The soluble portion was removed by filtration and the residue was washed with water. The extract and washings were combined and saturated Ba(OH)₂ solution (5 ml.) was added (Meier, 1958). The precipitate of crude glucomannan that formed was allowed to settle for 24 hr., centrifuged, dissolved in 4% (w/v) NaOH solution (2 ml.) at room temperature and reprecipitated with saturated Ba(OH)₂ solution (1 ml.). After 14 hr. the reprecipitated glucomannan was centrifuged, dissolved in 2N-acetic acid (4 ml.) and precipitated with ethanol. This repurified glucomannan fraction was centrifuged, washed with ethanol, dried and weighed.

Ethanol was added to the alkaline extracts, which were not precipitated by the addition of Ba(OH)₂ solution, until its concentration exceeded 80%. The resulting precipitate (hemicellulose fraction II) was allowed to settle for 24 hr.,

centrifuged, washed with ethanol, dried and weighed (Fig. 1). The alkali-insoluble residue (α -cellulose fraction) was washed with water, 2N-acetic acid and ethanol, dried and weighed.

Determination of the degree of polymerization of α -cellulose preparations

Nitration of the α -cellulose fraction. The α -cellulose fraction (100 mg.) was nitrated for 1 hr. at room temperature with HNO₃-H₃PO₄-P₂O₅ (70:50:57, by wt.) (10 ml.). The nitrated α -cellulose fraction was immersed in ice-water (100 ml.) for 5 min., in 2% (w/v) Na₂CO₃ solution (100 ml.) for 5 min. and then washed over a period of 3 days at 5° with water which was frequently changed (Atchison, 1943). The nitrated α -cellulose fraction was dried at 0.1 mm. Hg over P₂O₅.

Reprecipitated nitrated material was obtained by adding an equal volume of water to a 1% (w/v) solution of the nitrocellulose in acetone. The precipitated fraction was filtered and dried at 0.1 mm. Hg. over P₂O₅.

Determination of the degree of polymerization. A known weight (approx. 100 mg.) of the nitrated fraction was dissolved in acetone (100 ml.) and the viscosity of the solution at 25° measured in an Ostwald viscometer. Measurements at several dilutions were made. From a graph showing the variation of η_{sp}/c with c , the intrinsic viscosity, $[\eta]$, was determined by extrapolation to zero concentration (c is concentration of nitrocellulose fraction in g./100 ml., $\eta_{sp} = \eta_a/\eta_0 - 1$, where η_a is viscosity of solution and η_0 is viscosity of solvent).

The following formula (Harland, 1959) was used to calculate the degree of polymerization (D.P.):

$$[\eta] = 0.0142(\text{D.P.})^{0.533}$$

RESULTS

Sycamore (*Acer pseudoplatanus*) and pine (*Pinus sylvestris*) were chosen for this investigation since these species allowed a comparison between an angiosperm and a gymnosperm to be made.

The microscopical examination of transverse sections of the sapling stems (Plate 1) showed that the regions isolated and from which the polysaccharides were prepared corresponded to the cambium, the phloem and the xylem of the original stem and also corresponded to the regions previously investigated (Thorner & Northcote, 1961a, b).

In order to compare the composition of the cell walls in the different regions of each tree, it was necessary to compare the percentage composition of extractive-free material since the analyses of whole tissues included the cell contents. The weight of each polysaccharide and of lignin was corrected for the mineral matter present and expressed as a percentage of the extractive-free material (Table 1).

The carbohydrate composition of each polysaccharide preparation is shown in Table 2. These results were calculated as the percentage of the total sugar estimated in each hydrolysate after

Plate 1. Transverse section of stem of pine sapling stained with safranin and picric aniline blue. The line *a-a* indicates the region where lignification can first be detected by the safranin stain and the line *b-b* marks the region at which the walls stain completely red. *A*: Low magnification to show general distribution of tissues. The area marked in the rectangle indicates the regions shown in *B*, *C* and *D*. *B*: Intact tissue showing phloem, cambium and xylem. *C*: After removal of bark. *D*: After removal of cambium.

correction for losses during hydrolysis (Thorner & Northcote, 1961b) and after expression as anhydro sugar units. In Table 2 each anhydro sugar has been designated as a hexosan or pentosan; this is

not meant to imply that they existed as distinct polysaccharides.

A quantitative estimation was made of the methoxyl groups in each xylan fraction. From

Table 1. *Percentage composition of the extractive-free material in each region of the two trees*

All polysaccharide fractions are corrected for the mineral matter present.

Region of tree ...	Pine			Sycamore		
	Phloem	Cambium	Xylem	Phloem	Cambium	Xylem
Pectic substances	6.0	12.0	2.0	3.0	14.1	4.5
Lignin	49.6	4.0	28.5	49.0	11.7	26.0
α -Cellulose fraction	20.5	39.2	33.2	26.6	36.2	38.7
Xylan fraction	2.8	8.6	1.7	8.6	8.6	13.0
Glucomannan fraction	0.2	0.2	1.4	0.6	0.4	0.5
Hemicellulose fraction I	12.2	27.8	14.5	8.0	25.5	11.5
Hemicellulose fraction II	2.8	8.7	18.7	1.1	2.4	2.2

Table 2. *Percentage composition of the polysaccharide fractions from each region of the two trees*

The designation hexosan or pentosan is not meant to imply that they existed as distinct polysaccharides.

Region of tree ...	Pine			Sycamore		
	Phloem	Cambium	Xylem	Phloem	Cambium	Xylem
α -Cellulose fraction						
Galactan	0.0	4.0	0.0	Trace	0.0	0.0
Glucan	89.7	87.7	84.2	88.4	99.1	96.5
Mannan	0.0	3.0	12.7	0.0	0.0	Trace
Araban	9.6	5.3	0.0	5.3	0.3	0.0
Xylan	0.7	0.0	3.1	6.3	0.6	3.5
Xylan fraction						
Galactan	6.8	22.7	4.8	8.0	4.8	0.0
Glucan	23.0	3.3	2.2	10.7	0.0	18.7
Araban	27.5	26.7	12.4	12.9	7.3	Trace
Xylan	38.3	43.6	65.6	49.6	77.3	69.2
Uronic anhydride	5.8	5.0	14.8	19.3	10.3	12.6
4-O-Methylglucuronic acid anhydride	4.3	3.9	15.0	18.6	10.6	12.2
Starch	+	Trace	Trace	+	Nil	+
Glucomannan fraction						
Galactan	11.5	Trace	3.6	17.8	21.9	0.0
Glucan	10.1	33.5	23.3	39.4	31.8	20.4
Mannan	39.8	57.6	61.8	28.1	17.1	39.8
Araban	22.3	3.0	3.3	8.8	9.2	0.0
Xylan	13.5	6.0	6.5	2.2	17.9	33.2
Uronic anhydride	2.0	Trace	1.5	0.8	Trace	3.9
Starch	Nil	+	Nil	Nil	Nil	Nil
Hemicellulose fraction I						
Galactan	22.3	18.4	5.6	12.7	5.8	2.8
Glucan	18.0	14.0	9.0	22.1	0.0	8.9
Mannan	5.6	2.7	15.0	0.0	0.0	0.0
Araban	24.6	26.7	9.1	13.6	11.6	1.6
Xylan	26.0	34.2	50.7	37.8	70.4	73.8
Uronic anhydride	3.9	6.0	9.6	12.4	9.2	11.4
Starch	+	+	+	+	Nil	+
Hemicellulose fraction II						
Galactan	3.5	6.2	4.0	9.2	4.6	0.0
Glucan	31.9	21.7	15.8	28.0	19.8	30.4
Mannan	50.0	64.6	25.5	32.5	24.1	51.0
Araban	4.9	4.1	14.5	13.9	5.6	0.0
Xylan	8.7	3.2	33.0	11.6	40.8	15.9
Uronic anhydride	1.1	Trace	7.2	5.2	3.8	3.2
Starch	Nil	Nil	Nil	+	Trace	Nil

Table 3. *Molar ratios of xylose to 4-O-methylglucuronic acid in the xylan preparations, and of mannose to glucose in the glucomannan preparations*

Figures in parentheses refer to the ratios in the soluble hemicellulose fractions II (Fig. 1).

	Pine			Sycamore		
	Phloem	Cambium	Xylem	Phloem	Cambium	Xylem
Xylose/4-O-methylglucuronic acid	12.9	16.2	6.3	3.9	10.6	8.2
Mannose/glucose	3.9 (1.6)	1.7 (2.9)	2.7 (1.6)	0.7 (1.2)	0.5 (1.2)	1.9 (1.7)

Table 4. *Intrinsic viscosities $[\eta]$ and degrees of polymerization (D.P.) of α -cellulose preparations*

		Pine			Sycamore		
		Phloem	Cambium	Xylem	Phloem	Cambium	Xylem
Initial nitrated α -cellulose preparations	$[\eta]$	6.4	8.0	5.8	7.6	8.6	10.0
	D.P.	695	890	630	840	965	1150
Reprecipitated nitrated α -cellulose preparations	$[\eta]$	7.8	8.7	7.6	11.2	9.9	11.7
	D.P.	870	975	840	1260	1125	1325

these results the percentage of 4-O-methylglucuronic acid anhydride was calculated (Table 2). The close agreement of these values with those obtained by estimating the total uronic anhydride content by the method of Dische (1947) (Table 2) confirmed the chromatographic evidence that only traces of galacturonic acid and no glucuronic acid were present in the xylan preparations. The molar ratio of xylose to 4-O-methylglucuronic acid in each xylan preparation was calculated (Table 3).

Hagglund *et al.* (1956) showed that if a gentle method was used for the extraction of lignin from woody tissues then treatment of the holocellulose with a technical-grade dimethyl sulphoxide gave a hemicellulose which contained the majority of the acetyl groups present in the original wood. We found that our xylan preparations contained no acetyl groups, although these were present in the original extractive-free material. It was shown that when the technical grade dimethyl sulphoxide, which had been used for the xylan preparations, was added to cellulose acetate, almost all the acetyl groups were removed from the polymer in 24 hr.

The carbohydrate composition of the glucomannan preparations is shown in Table 2. In order to compare the glucomannan fractions in the different regions of the two trees, the molar ratio of mannose to glucose was calculated for both the glucomannan fractions and the hemicellulose fractions II (Fig. 1) and are shown in Table 3.

Two properties of the α -cellulose fractions, their carbohydrate composition (Table 2) and degree of polymerization (Table 4) have been compared. Some recent investigations (Snyder & Timell, 1955; Timell, 1956, 1957) have indicated that the chain length of the celluloses in the xylem of angiosperms and gymnosperms was about 5000 glucose units; our results, however, indicated a chain length of about 1000. In order to check the

measurements a determination was made of the degree of polymerization of the cellulose in cotton fibres, which is known to be 6000–10000 (Gralén & Svedberg, 1943; Hessler, Merola & Berkley, 1948; Timell, 1955). The cellulose was prepared from Sudan Sakel cotton (kindly given by The Shirley Institute, Didsbury, Manchester 20) by extracting the non-cellulosic constituents with ethanolamine at 150° for 3 hr. (Tripp, Moore & Rollins, 1951). The degree of polymerization of this cellulose was found to be 7800. The α -cellulose preparations contained varying percentages of non-glucan material (Table 2). Removal of these by repurification of the nitrated α -cellulose fraction resulted in an increased degree of polymerization (Table 4).

DISCUSSION

The investigation of the composition of a cell at different stages of its development can give a considerable amount of information about the biosynthetic mechanisms occurring during the cell's growth. The development of secondary thickened xylem cells from cambial cells in trees has been studied in this way (Thorner & Northcote, 1961*a, b*). These investigations indicated that the hemicellulose component of the primary cell walls in the cambial region contained a smaller percentage of 4-O-methylglucuronic acid anhydride than the hemicellulose synthesized during secondary thickening. In higher plants the only reported occurrence of 4-O-methylglucuronic acid has been as a constituent of the xylan component of the cell wall (Aspinall, 1959). Therefore the molar ratio of xylose to 4-O-methylglucuronic acid in the xylan component of the cambial cell wall should be greater than the ratio in the xylan component of secondary thickened xylem tissues; this was found to be true (Table 3). It was not possible to ascertain

whether the difference was due to the addition of 4-*O*-methylglucuronic acid units on to similar xylan chains in both the primary and secondary cell walls during the secondary thickening of the cambial cell, or whether the xylan synthesized during secondary thickening contained a larger percentage of this uronic acid than the xylan deposited in the primary cell wall. The absence of any unsubstituted glucuronic acid in any xylan fraction hydrolysate indicated that the addition of 4-*O*-methylglucuronic acid probably occurred by the addition of the methylated uronic acid and not by the addition of glucuronic acid followed by methylation.

The molar ratio of xylose to 4-*O*-methylglucuronic acid in the xylan fractions prepared from the xylem of the gymnosperm (6.3:1) and the angiosperm (8.2:1) are similar to those reported for pine and maple wood (Garegg & Lindberg, 1960; Timell, 1959). No previous reports have been made on the structure of xylyans in primary cell walls.

Dimethyl sulphoxide proved to be a more specific reagent for the extraction of the xylan component than did potassium hydroxide solution, but it did not remove all the xylan component from the cell-wall preparations (Tables 1 and 2). However, in all the polysaccharide preparations from one region of a tree, the ratio of xylose to uronic anhydride was the same, and hence the dimethyl sulphoxide extracted a portion of the xylan component which was typical of the whole. Despite the more specific extraction procedure, the xylan preparations still contained other polysaccharides (galactans, glucans and possibly some arabans) (Table 2). In certain cases some of the arabinose residues may be covalently linked to the xylan chain as already shown for the xylan from the wood of pine (Garegg & Lindberg, 1960).

Very little is known about the structure of the xylyans in the phloem tissue of trees. Mian & Timell (1960b) showed that the inner bark of birch (*Betula papyrifera*) contained a xylan that had an almost identical composition to that found in the wood (molar ratio of xylose to 4-*O*-methylglucuronic acid was 10:1), and Painter & Purves (1960) found that the xylan in the inner bark of spruce (*Picea glauca*) contained much fewer 4-*O*-methylglucuronic acid residues than the xylan in the wood. Our results showed that the xylan fraction in the sycamore phloem contained twice the number of 4-*O*-methylglucuronic acid residues for a given number of xylose units than the xylan fraction of the xylem, whereas for the pine the phloem xylan fraction contained only half as many uronic acid residues as that of the xylem (Table 3). Therefore during the differentiation of a phloem cell from a cambial initial in the gymnosperms fewer 4-*O*-methylglucuronic acid residues were added to

the xylyans than during the development of a xylem cell; the reverse occurred in the angiosperms.

Meier (1958) has shown that the polysaccharides which contain mannose can be selectively precipitated from a sodium hydroxide extract of cell walls of woody tissues by the addition of barium hydroxide solution. If this selective precipitation occurred then the ratio of mannose to glucose in the material precipitated should be greater than or equal to that of the material remaining in solution. In the investigation reported here this only occurred with the extracts from the xylem tissues of the two trees and from the phloem of the pine (Table 3). In the glucomannan precipitates in which the ratio was lower than that in the soluble hemicellulose fraction II, glucans were probably also precipitated by the addition of the barium hydroxide solution. Starch was only detected in the glucomannan precipitate from the cambial region of the pine (Table 2), therefore glucans other than starch were probably present in the glucomannan fractions prepared from the other two regions. The presence of β -glucans in phloem tissues has been indicated by some previous investigations (Painter & Purves, 1960; Thorner & Northcote, 1961b). Those hemicellulose fractions II (Fig. 1) which contained a higher mannose to glucose ratio than that which occurred in the glucomannan precipitates were probably more representative of the composition of the glucomannan component of the cell wall than the contaminated glucomannan precipitates. Comparisons of the composition of the glucomannan components in cambium with that in xylem and phloem tissues in the pine were therefore best made between the glucomannan precipitates of the differentiated tissues and the soluble hemicellulose fraction II of the cambial region. This indicated that the glucomannan synthesized during secondary thickening in xylem had a composition very similar to that deposited in the primary cell wall. The glucomannan component of phloem cell walls contained a higher percentage of mannose. Similar comparisons in the sycamore were not possible since the soluble hemicellulose fraction II which contained the higher mannose to glucose ratio in the phloem and cambial regions also contained starch (Table 2). The ratio in the glucomannan precipitates from the xylem regions of the two trees are identical with those reported for glucomannans in other hardwoods (Timell, 1960) and softwoods (Timell, 1961). No previous investigations have been made of the structure of the glucomannans in any other tissue of the tree than the xylem.

The α -cellulose preparations contained sugars other than glucose (Table 2) and if these occurred as low-molecular-weight polysaccharides distinct from the major glucan component then the degree

of polymerization found for these preparations would be lower than that of the glucan. However, the percentages of material other than glucose were small and approximately the same in the preparations made from the phloem, cambium and xylem of each tree. Therefore comparative studies were possible without further fractionation to obtain the higher-molecular-weight material. No significant difference could be detected between the degree of polymerization of the preparation of α -cellulose from the various regions of each tree (Table 4). When the lower-molecular-weight components were removed by reprecipitation of the nitrated α -cellulose fraction the small differences between each region were even decreased (Table 4).

Other investigations with cotton fibres (Hessler *et al.* 1948) have shown that the chain length of cellulose in the primary cell walls was less than that in secondary thickened walls, but no previous investigations on the primary and secondary cell walls of trees have been reported. Differences in the molecular weight of α -celluloses prepared from the inner phloem and the wood of birch have been found by Mian & Timell (1960*a*).

Thorner & Northcote (1961*a, b*) found that the composition of the cambial cell wall differed considerably from that of the xylem and phloem walls in both hardwoods and softwoods and that there was no similarity between the fully developed walls in both these classes of tree. However, the cambial cell walls from angiosperms and gymnosperms were very similar and this is again observed in the more detailed analyses (Fig. 1) described in this paper (Tables 1 and 2).

The differences in the composition of the pectic substances prepared from each region of the two trees have been reported by Thorner & Northcote (1961*b*).

SUMMARY

1. The xylan, glucomannan and α -cellulose fractions of the phloem, cambial and xylem tissues of pine (*Pinus sylvestris*) and sycamore (*Acer pseudoplatanus*) saplings have been prepared.

2. The percentage of 4-*O*-methylglucuronic acid in the xylan fraction isolated from the primary cell walls of the cambial region was lower than that in the xylan fractions obtained from differentiated tissues. The significance of this observation on the biosynthesis of the xylans has been discussed.

3. The xylan in the sycamore phloem contained twice the number of 4-*O*-methylglucuronic acid residues for a given number of xylose units than the xylan of the xylem, whereas for the pine the phloem xylan contained only half as many uronic acid residues as that of the xylem.

4. The composition of the glucomannan deposited during secondary thickening in the pine

was similar to that deposited in the primary cell wall, whereas the glucomannan fraction deposited during the formation of a phloem cell was richer in mannose.

5. The degree of polymerization of the α -cellulose was almost identical in each region of the tree.

6. Further evidence has been obtained for the similar composition of the cambial cell walls in angiosperms and gymnosperms.

We wish to thank Mr A. Bassett of the Forestry Commission for the pine saplings and Dr R. Hill for providing the sycamore saplings. J. P. T. gratefully acknowledges the receipt of a grant from the Agricultural Research Council during the tenure of which this work was carried out.

REFERENCES

- Aspinall, G. O. (1959). *Advanc. Carbohydr. Chem.* **14**, 429.
 Atchison, J. E. (1943). *Paper Tr. J.* **116**, 23.
 Dische, Z. (1947). *J. biol. Chem.* **167**, 189.
 Garegg, P. J. & Lindberg, B. (1960). *Acta chem. scand.* **14**, 871.
 Gralén, N. & Svedberg, T. (1943). *Nature, Lond.*, **152**, 625.
 Hagglund, E., Lindberg, B. & Macpherson, J. (1956). *Acta chem. scand.* **10**, 1160.
 Harland, W. G. (1959). In *Recent Advances in the Chemistry of Cellulose and Starch*, p. 271. Ed. by Honeyman, J. London: Heywood and Co. Ltd.
 Hessler, L. E., Merola, G. V. & Berkley, E. E. (1948). *Text. Res. J.* **18**, 628.
 Johansen, D. A. (1951). In *Manual of Phycology*, appendix B. Ed. by Smith, G. M. Waltham, Mass.: Chronica Botanica Co.
 Jones, J. K. N. & Painter, T. J. (1957). *J. chem. Soc.* p. 669.
 Meier, H. (1958). *Acta chem. scand.* **12**, 144.
 Mian, A. J. & Timell, T. E. (1960*a*). *Canad. J. Chem.* **38**, 1191.
 Mian, A. J. & Timell, T. E. (1960*b*). *T.A.P.P.I.* **43**, 775.
 Painter, T. J. & Purves, C. B. (1960). *T.A.P.P.I.* **43**, 729.
 Pregl, F. (1937). In *Quantitative Organic Microanalysis*, p. 171. Ed. by Roth, H. London: J. and A. Churchill Ltd.
 Ritter, G. J., Seborg, R. M. & Mitchell, R. C. (1932). *Industr. Engng Chem. (Anal.)* **4**, 202.
 Snyder, J. L. & Timell, T. E. (1955). *Svensk Papp-Tidn.* **59**, 857.
 Thorner, J. P. & Northcote, D. H. (1961*a*). *Biochem. J.* **81**, 449.
 Thorner, J. P. & Northcote, D. H. (1961*b*). *Biochem. J.* **81**, 455.
 Timell, T. E. (1955). *Industr. Engng Chem.* **47**, 2166.
 Timell, T. E. (1956). *Svensk Papp-Tidn.* **59**, 1.
 Timell, T. E. (1957). *T.A.P.P.I.* **40**, 25.
 Timell, T. E. (1959). *Canad. J. Chem.* **37**, 893.
 Timell, T. E. (1960). *Svensk Papp-Tidn.* **63**, 472.
 Timell, T. E. (1961). *T.A.P.P.I.* **44**, 88.
 Tripp, V. W., Moore, A. T. & Rollins, M. L. (1951). *Text. Res. J.* **21**, 886.
 Whistler, R. L. & Jeanes, A. (1943). *Industr. Engng Chem. (Anal.)* **15**, 317.
 Wise, L. E., Murphy, M. & D'Addieco, A. A. (1946). *Paper Tr. J.* **122**, 35.