Inositol Metabolism in Plants. III. Conversion of Myo-inositol-2-3H to Cell Wall Polysaccharides in Sycamore (Acer pseudoplatanus L.) Cell Culture

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Summary. Prolonged growth of cell cultures of sycamore (Acer pseudoplatanus L.) on agar medium containing myo-inositol-2-3H resulted in incorporation of label predominately into uronosyl and pentosyl units of cell wall polysaccharides. Procedures normally used to distinguish between pectic substance and hemicellulose yielded carbohydrate-rich fractions with solubility characteristics ranging from pectic substance to hemicellulose yet the uronic acid and pentose composition of these fractions was decidedly pectic. Galacturonic acid was the only uronic acid present in each fraction. Subfractionation of alkali-soluble (hemicellulosic) polysaccharide by neutralization followed by ethanol precipitation gave 3 fractions, a water-insoluble, an ethanol-insoluble, and an ethanol-soluble fraction, each progressively poorer in galacturonic acid units and progressively richer in arabinose units; all relatively poor in xylose units.

Apparently, processes involved in biosynthesis of primary cell wall continued to produce pectic substance during cell enlargement while processes leading to biosynthesis of typically secondary cell wall polysaccharide such as 4-O-methyl glucuronoxylan were not activated.

Myo-inositol, supplied exogenously, is readily used by intact and detached plant tissues as a source of uronosyl and pentosyl units in cell wall polysaccharides (21). This observation is now extended to include cell cultures of sycamore (Acer pseudoplatanus L.) derived from cambial cell tissue (19, 20). Some cultures exhibit a partial or complete requirement for myo-inositol (1, 10, 25, 37), but this requirement may be altered in response to pathological or nutritional changes (7, 38). Although sycamore cells grow well on a defined medium containing myo-inositol, strains have appeared in our cultures during the past 4 years that are capable of sustained growth in the absence of added myo-inositol. Since very little is known regarding the fate of myo-inositol and this information is essential if work is to proceed on strains which exhibit an independence on external sources for this cyclitol, we have undertaken an investigation into the fate of myo-inositol-2-3H in cells grown continuously for 10 weeks on an agar medium containing this label. Our results reveal that myo-inositol is readily converted to pectic substance in cell wall polysaccharides of sycamore. Moreover, we find that the entire non-cellulosic polysaccharide of these cells has a monosaccharide composition characteristic of primary cell wall substance.

Materials and Methods

Tissue Culture. Sycamore callus on agar was obtained from Dr. P. Albersheim. These cells originated in a clone first isolated by Drs. D. T. A. Lamport and D. H. Northcote (20). Cultures used in this study have been maintained on the agar medium of Murashige and Skoog (27) since 1962. A casein hydrolysate (Edamin, Sheffield Chemical Company) was routinely included in the medium. When old callus was transferred to fresh agar medium, care was made to select pale yellow or colorless regions of old callus for the transfer. Three pieces (50 to 100 mg, fr wt) were transferred aseptically to a 250 ml Erlenmeyer flask containing 100 ml of freshly prepared medium. In labeled experiments, 1 mg of myo-inositol-2-3H (24) with a specific activity of 30 μc/mg was present in each flask in lieu of unlabeled myo-inositol. Cultures were held at 27° in subdued light. At the end of a selected growth period, clones were removed, freed of adhering agar, and weighed.

Autoradiography. Tissues were fixed overnight in acetic acid:ethanol (1:2, v/v), dehydrated, and embedded in paraffin. Sections 6 μ thick were transferred to gelatin-coated slides, deparaffined and stained with periodic acid-Schiff reagent (PAS) (17) prior to autoradiography. Kodak AR 10 stripping film was applied by conventional procedure, exposed for 6 days, and developed. After development, sections were lightly stained (18) and examined.

1 Supported by grant No. GM-12422, United States Public Health Service.
Extractive Procedures for Carbohydrate. Fresh tissues were ground with ice-cold 80% (v/v) ethanol in an all-glass Tenbroeck homogenizer and centrifuged. The process was repeated 4 times with fresh ethanol to remove all traces of soluble radioactivity. The final residue was extracted with 100% ethanol, a mixture of chloroform:methanol (2:1, v/v), and ether. The combined 80% ethanol extracts are referred to as the ethanol-soluble fraction; the final residue as the ethanol-insoluble fraction.

Successive extractions with water, a combination of 0.1% (w/v) disodium EDTA and 0.2% (w/v) fungal pectinase (Rohm and Haas Pectinol R-10 concentrate), and, finally, strong alkali was used to solubilize carbohydrate present in the ethanol-insoluble residue. In the first step (extract I), 50 mg of residue was triturated in 10 ml of water for 10 minutes at 25°C. Insoluble residue was centrifuged, washed with water, dried, and weighed. This residue was resuspended in 10 ml of 0.1% EDTA plus 0.2% pectinase, adjusted to pH 4 with acetic acid and held for 6 hours at 37°C. A drop of toluene was added to reduce bacterial growth. Again, unhydrolyzed residue was removed by centrifugation, washed, dried, and weighed. The pectinase extract (extract II) was freed of protein by passage through a column of Dowex 50 (H⁺) resin. The final extract was made in 2 ml of 10% (w/v) KOH for 24 hours at 28°C. Following centrifugation, the residue was washed with 1 ml of 10% KOH. Extract and wash were combined, adjusted to pH 4.5 with glacial acetic acid, and allowed to stand at room temperature for several hours. A white precipitate, extract IIIa, formed and was recovered. When sufficient ethanol was added to bring the solution to 90% (v/v) alcohol, a second precipitate, extract IIIb, formed and was recovered. A third carbohydrate fraction, extract IIIc, remained in the 90% ethanol and was recovered, free of salts, by gel filtration on Sephadex G-25 (Pharmacia Fine Chem., Inc.) after removal of ethanol. The final residue was digested with a crude cellulase preparation from Myrothecium verrucaria (30).

Ion Exchange Chromatography. Acidic constituents in the ethanol-soluble fraction or in hydrolysates of extracts from the ethanol-insoluble fraction were separated on a column (15 × 0.9 cm) of Dowex 1 × 8, 200 to 400 mesh (formate) exchange resin by elution with a formic acid gradient. Uniform flow was maintained by means of a LKB Model 4912A peristaltic pump fitted with 3.2 mm bore tubing. Ten ml fractions were collected in a Beckman Model 132 fraction collector modified to accommodate high flow rates.

Elution was performed in 2 steps, an initial gradient from water to 0.1 x formic acid followed by a steep gradient from 0.1 to 3 x formic acid. Gradients were controlled by the use of a Varigrad Model 3000 mixer (Phoenix Precision Instrument Company). This device contains 9 chambers of equal volume, each connected in series by a small port equipped with stopcock. Chamber 1 contained 250 ml of water. Chambers 2 and 3 contained 250 ml and chamber 4 contained 160 ml of 0.1 x formic acid. Chamber 8 contained 160 ml of 3.0 x formic acid. At zero time, stopcocks interconnecting chambers 1, 2, and 3 were opened. When these chambers had emptied, the second gradient was initiated by opening the stopcock interconnecting chambers 3 and 4 followed by opening all interconnecting stopcocks up to and including chamber 8. Approximate changes in formic acid concentration throughout this elution are plotted in figure 2 (below).

Separation and Assay of Carbohydrates. Polysaccharides and oligosaccharide fragments extracted from the ethanol-insoluble fraction were hydrolyzed by autoclaving samples in 2 x HCl in sealed tubes for 30 minutes at 121°C. Hydrolyzed samples were evaporated to dryness in a rotary evaporator at 30°C, taken up in a measured volume of water and applied to Whatman No. 1 paper for chromatography. Carbohydrate extracted by pectinase was chromatographed directly, without an intervening acid hydrolysis. Ethanol-insoluble residue resistant to all extractive procedures, composed primarily α-cellulose, was partially hydrolyzed with the crude cellulase preparation from Myrothecium verrucaria. The extract was passed through Dowex 50(H⁺) and chromatographed.

Monosaccharides were separated by descending chromatography in solvent A (ethyl acetate:pyridine:water, 8:2:1, v/v) (36) for 24 hours, or solvent B (benzene:isooctane:pyridine:water, 1:5:5:3, v/v) (12), a single phase at 25°C, for 16 hours. Solvent A separated mannose from arabinose. Uronic acids were separated with solvent C (ethyl acetate:pyridine:acetic acid:water, 5:5:1:3, v/v) (11) and solvent D (ethyl acetate:acetic acid:water, 9:2:2, v/v) (33). After separation, papers were sprayed with aqueous acetone containing silver nitrate, dried, and sprayed with alcoholic KOH (35). Contrast between developed spots and paper background was preserved by a dip in 10% (w/v) sodium thiosulfate.

Total carbohydrate, reducing sugars, uronic acids, and pentoses, were determined quantitatively in each fraction and extract. Total carbohydrate was measured with sulfonated α-naphthol reagent (9). Uronic acids produced a brownish color in this reagent and had only 50% the absorption of glucose at 555 nm. All values reported here have been corrected on the basis of uronic acid content to allow a direct comparison of total carbohydrate among samples containing varying amounts of uronic acid. Uronic acid was determined by carbazole reaction in the presence of borate (6). Pentose and uronic acid together were measured by reaction with orcinol (8). In selected cases, monosaccharides were eluted individually after paper chromatography and assayed for radioactivity.
and reducing sugar or uronic acid content. Reducing sugar was estimated by a modification of the Folin alkaline ferricyanide method (2).

Measurement of radioactivity in aqueous solutions containing tritiated material was made by adding 0.5 ml of an appropriately diluted sample to a glass vial containing 15 ml of a mixture of naphthalene:2,5-diphenyloxazole:dimethyl 1,4-bis-(5-phenyl-oxazolyl)benzene (100:7.5:0.25, w/v) in 1 liter of 1,4-dioxane. Samples were counted in a Packard Model 3000 liquid scintillation spectrometer. Efficiency was about 22%. Paper chromatograms were scanned for areas containing tritium with a Packard Model 7200 windowless, strip scanner equipped with a Geiger detector.

Results

Others have described growth characteristics of sycamore cambial cells in coconut milk medium (19) and the M-6 medium (5) of Torrey and Shigemura (34). In the present study, cells maintained on Murashige and Skoog's agar medium grew slowly for 2 weeks then entered a rapidly accelerating growth phase that ended in an extended period of decreasing growth accompanied by progressive darkening throughout the callus mass (fig 1). Up to 10 weeks of growth, cells remained viable and healthy. Differentiation other than cell enlargement was not detectable.

In labeling experiments, cell cultures were grown on 2 concentrations of myo-inositol, 1 and 5 mg/100 ml of medium. Both levels contained 1 mg of myo-inositol-2-3H/100 ml so that the specific activity of medium at the high level was one-fifth of the other. A total of 9 fragments (about 100 mg/fragment) of 7 week-old callus was transplanted to fresh medium at each myo-inositol concentration. In the 10 week growth period, average fresh weight increased 19-fold. Tritium uptake from the agar was 29% for the 1 mg level of myo-inositol and 32% for the 5 mg level. Similar patterns of tritium distribution among carbohydrate fractions were found for both concentrations. Most of the studies were restricted to fractions obtained from tissue supplied with 1 mg myo-inositol/100 ml. Results of tritium distribution between ethanol-soluble and ethanol-insoluble fractions (table 1) refer to 0.152 g of dry residue recovered from 6.76 g of fresh tissue after exhaustive extraction with ethanol, and finally chloroform:methanol and ether. Negligible label was removed as lipid.

Identification of Glucuronic Acid in the Ethanol-

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Total radioactivity $10^3$ cpm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol-soluble</td>
<td>6630 (48% of $^3$H in cells)</td>
</tr>
<tr>
<td>Recovered on Dowex 50(H+) resin</td>
<td>negligible</td>
</tr>
<tr>
<td>Recovered on Dowex 1 (formate) resin</td>
<td>930</td>
</tr>
<tr>
<td>Present in neutral components</td>
<td>5700</td>
</tr>
<tr>
<td>Ethanol-insoluble</td>
<td>7320 (52% of $^3$H in cells)</td>
</tr>
<tr>
<td>Extract I (water)</td>
<td>720</td>
</tr>
<tr>
<td>Extract II (EDTA-pectinase)</td>
<td>3330</td>
</tr>
<tr>
<td>Extract III (alkali)</td>
<td>2970</td>
</tr>
<tr>
<td>Final residue</td>
<td>390</td>
</tr>
</tbody>
</table>

Table I. Tritium Distribution in Sycamore Cells Grown on myo-inositol-2-3H

Fig. 1. Growth of sycamore cambial cells on agar medium of Murashige and Skoog.

Fig. 2. Gradient elution from Dowex 1 (formate) resin of labeled acidic components present in ethanol-soluble fraction.
Soluble Fraction. When the ethanol-soluble fraction was brought to dryness in a rotary evaporator at 30° and redissolved in water, practically all tritium originally present was recovered as water soluble material. Little, if any, tritium was lost by exchange processes. The aqueous solution was passed through Dowex 50(H+) resin with negligible loss of radioactivity, transferred to a Dowex 1(formate) resin, washed with water until the effluent was free of tritium, and then eluted from Dowex 1 resin with formic acid. Tritium in the neutral effluent chromatographed in solvent A as myo-inositol. Further characterization was not attempted.

Elution of Dowex 1(formate) resin with formic acid produced 3 radioactive peaks (fig 2). Only the third, between 400 to 520 ml was identified. Individual fractions under this peak were combined, taken to dryness, dissolved in water, and adjusted above pH 7 with Ca(OH)2. To this solution was added 101 mg of unlabeled Ca p-glucuronate. After complete solution of the added glucuronate, sufficient ethanol was added to initiate crystallization. Of the tritium recovered from fractions under the peak, 90% appeared in the crystalline Ca salt (table II). Recrystallization did not alter the specific activity. Identity was established by converting the Ca salt to its free acid with Dowex 50(H+) resin, reducing the free glucuronic acid to gulonic

Table II. Specific Activities of Ca Glucuronate and its Derivatives after Recovery from the Ethanol-Soluble Fraction

<table>
<thead>
<tr>
<th>Product*</th>
<th>Specific activity cpm/μmole</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca p-glucuronate**</td>
<td></td>
</tr>
<tr>
<td>1st crystallization</td>
<td>10.5</td>
</tr>
<tr>
<td>2nd crystallization</td>
<td>10.6</td>
</tr>
<tr>
<td>L-Gulono-γ-lactone</td>
<td>9.4</td>
</tr>
<tr>
<td>L-Gulonamide</td>
<td>10.7</td>
</tr>
</tbody>
</table>

* Radioactivity recovered from Dowex 1 (formate) eluate fractions between 400 to 520 ml was 5720 cpm.
** Diluted with 100.8 mg of unlabeled Ca glucuronate.

Table III. Radioactivity and Composition of Cell Wall Fractions in the Ethanol-insoluble Fraction

<table>
<thead>
<tr>
<th>Extract</th>
<th>Residue extracted</th>
<th>Carbohydrate* mg</th>
<th>Counts* 10^-3 cpm</th>
<th>Distribution of radioactivity after hydrolysis*</th>
<th>Monosaccharide composition** %</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>5.5***</td>
<td>5</td>
<td>240</td>
<td>UA 57 A 35 X 13 15 Gal 29 G 9 M</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>14.2***</td>
<td>12</td>
<td>1110</td>
<td>57 32 9 33 14 5 22 26</td>
<td></td>
</tr>
<tr>
<td>IIIa</td>
<td>2.6</td>
<td>1</td>
<td>50</td>
<td>81 11 3 46 6 14 12 22</td>
<td></td>
</tr>
<tr>
<td>IIIb</td>
<td>9.6</td>
<td>7.5</td>
<td>400</td>
<td>31 57 12 22 27 9 19 14 19 4</td>
<td></td>
</tr>
<tr>
<td>IIIc</td>
<td>9.2***</td>
<td>2</td>
<td>620</td>
<td>7 89 4 80 80 80 80 80 80 80</td>
<td></td>
</tr>
<tr>
<td>Final residue</td>
<td>10.9</td>
<td>130</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* All values reported in this table refer to an initial sample of 50 mg of ethanol-insoluble material.
** Uronic acid (UA), arabinose (A), xylose (X), galactose (Gal), glucose (G), and mannose (M).
*** Values obtained by difference in wt of residue before and after extraction.
† Trace.
‡ Not determined.

Ethanol-insoluble Residue. Successive extractions with water, EDTA plus pectinase, and alkali solubilized nearly all tritium (table I) and about 80% of the total mass of residue (table III). Comparison of columns 2 and 3 in table III reveal that each extraction removed substance other than carbohydrate. This is most evident in extract IIIc where only 2 mg of the 9.2 mg of substance soluble in 90% (v/v) ethanol after alkaline extraction reacted positively with sulfonated α-naphthol. Values in these 2 columns represent recovery from 50 mg of residue containing 24 × 10^6 cpm.

Water removed polysaccharides rich in hexose (47% of total carbohydrate extracted). All label extracted was recovered in uronic acid and pentose, none in hexose.

Pectinase-EDTA extraction solubilized over one-third of the carbohydrate and 50% of the tritium remaining in the residue following extraction with water. EDTA alone was no more effective than pectinase alone (fig 3). Combined, the effect was more than additive. Extraction was limited to 6 hours since most radioactivity released by this treatment occurred in the first few hours. Others have used a combination of pectinase and EDTA (26) but failed to note its synergistic effect.

Both pectinase-EDTA and alkali released carbohydrate with uronic acid and pentose compositions roughly parallel to the tritium content of these constituents. Unlabeled hexoses reduced percentage compositions but failed to alter relative ratios between uronic acid and pentose. The uronic acid was exclusively galacturonic acid in the pectinase-EDTA extract. This was established by its elution characteristics from Dowex 1(formate) resin as well as by paper chromatography in solvents C and D.

Alkali solubilized about 21 mg of the pectinase-EDTA resistant residue but only half of this was acid with NaBH4, lactonizing the reduced product, and converting it to its amide (23) with no significant change in specific activity.

Identification of Constituents in Ethanol-insoluble Residue. Successive extractions with water, EDTA plus pectinase, and alkali solubilized nearly all tritium (table I) and about 80% of the total mass of residue (table III). Comparison of columns 2 and 3 in table III reveal that each extraction removed substance other than carbohydrate. This is most evident in extract IIIc where only 2 mg of the 9.2 mg of substance soluble in 90% (v/v) ethanol after alkaline extraction reacted positively with sulfonated α-naphthol. Values in these 2 columns represent recovery from 50 mg of residue containing 24 × 10^6 cpm.

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<tr>
<td>Final residue</td>
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<td>130</td>
<td></td>
<td></td>
<td></td>
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* All values reported in this table refer to an initial sample of 50 mg of ethanol-insoluble material.
** Uronic acid (UA), arabinose (A), xylose (X), galactose (Gal), glucose (G), and mannose (M).
*** Values obtained by difference in wt of residue before and after extraction.
† Trace.
‡ Not determined.
Since extract IIIb contained galacturonic acid, it was of interest to learn if this substance was attacked by pectinase-EDTA. Hydrolysis followed the same conditions already described. After hydrolysis, the extract was passed through a column of Dowex 50(H+) resin, then through Dowex 1(formate) resin. About one-third of the tritium remained on the latter column. Elution with the prescribed 0.1 N formic acid gradient produced a single peak in the galacturonic acid region (fig. 4). Higher oligomers of galacturonic acid were eluted by extending the gradient to 3 N formic acid. Fractions corresponding to the area under the first peak were combined, taken to dryness in a rotary evaporator at 30°, redissolved in a small volume of water containing 59.6 mg of Na Ca p-galacturonate and left to crystallize. After a second crystallization

![Graph](https://example.com/graph.png)

**Fig. 3.** Release of tritium from ethanol-insoluble fraction with water (▲), EDTA (△), pectinase (●), or pectinase plus EDTA (○).

reactive to sulfonated α-naphthol. Neutralization with acetic acid caused a small volume of material, extract IIIa, to precipitate. It was composed mainly of uronic acid and hexose units. The uronic acid was identified as galacturonic acid by chromatography in solvents C and D.

Following removal of extract IIIa and addition of 10 volumes of ethanol to the neutral supernatant, a second precipitate formed (extract IIIb). It was recovered as a water-soluble, white product. Acid hydrolysis gave a uronic acid, arabinose, galactose, and glucose as major constituents with smaller amounts of xylose and mannose. The uronic acid had chromatographic properties of galacturonic acid. Since it was most surprising to find only galacturonic acid rather than glucuronic acid or 4-o-methyl glucuronic acid in this alkali-extracted fraction, a more detailed characterization was made (28).

A portion of hydrolyzed extract IIIb was separated on paper with solvent C. The uronic acid was eluted and transferred to a column of Dowex 1(formate). Upon elution with formic acid, all radioactivity appeared in fractions corresponding to galacturonic acid. Reduction of this radioactive material with NaBH₄ gave a single product with the chromatographic properties of galactonic acid and galactonolactone.

**Table IV.** Specific and Total Radioactivity Recovered as Galacturonic Acid and its Derivatives from Extract IIIb of the Ethanol-insoluble Fraction

<table>
<thead>
<tr>
<th>Product</th>
<th>Specific activity cpm/μmole</th>
<th>Total activity cpm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Area under 1st peak (Dowex 1 resin)</td>
<td></td>
<td>approx. 9000</td>
</tr>
<tr>
<td>Recovery as Na Ca p-galacturonate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st crystallization</td>
<td>31</td>
<td>7300*</td>
</tr>
<tr>
<td>2nd crystallization</td>
<td>37</td>
<td>8800*</td>
</tr>
<tr>
<td>Conversion to L-galactonamide</td>
<td>40</td>
<td>9500*</td>
</tr>
</tbody>
</table>

* Specific activities are calculated on the basis of dilution with 59.6 mg of Na Ca p-galacturonate.
from water and alcohol, it was reduced to L-galactonic acid with NaBH₄, lactonized, and converted to L-galactonamide. Each step increased the specific activity of the product (table IV). The L-galactonamide reached a value that accounted for all tritium recovered in pooled fractions under the peak.

The alcoholic supernatant from extract IIIb still retained over one-half of the tritium present in the alkaline extract as an orcinol-reactive carbohydrate. This solution was desalted on a Sephadex G-25 column (20 × 0.9 cm). Elution with water produced a single peak of radioactivity coincident with an orcinol-reactive peak (fig 5). One ml fractions under the peak, now free from salt, were hydrolyzed individually and examined by paper chromatography for monosaccharide. Each contained arabinose as its major constituent with a small quantity of galacturonic acid and traces of xylose, galactose, and glucose.

Another portion of extract IIIc, desalted, was placed on a column of Sephadex G-15 (75 × 1.2 cm) and eluted with water (fig 6). Radioactivity appeared as 3 peaks. The first was excluded by the gel (molecular weight > 1500), the second was only partially excluded, and the third, almost completely separated from the first 2 peaks, appeared in the region corresponding to mono- and disaccharides. Dialysis of extract IIIc for 18 hours against a large volume of water completely removed the peak corresponding to low molecular weight constituents but failed to alter the other 2 peaks appreciably (fig 6). Chromatography of constituents present in the dialyzable peak on thin layer cellulose plates revealed the presence of free arabinose as well as a series of slow-migrating radioactive components, possibly higher oligomers of arabinose and, perhaps, galacturonic acid.

**Microscopic Study.** Meristematic cells were absent in the tissue after 10 weeks of growth on agar medium. Cells varied in size but all were 20 μ or greater in diameter. They were vacuolated and thick-walled. Tissue masses were extremely friable. There was no evidence of differentiation into special cell types. Tissue slices of callus treated with PAS, contained middle lamellar regions more intensely stained than adjacent cell walls. Autoradiography (fig 7) revealed a pattern of tri-
Fig. 7. Autoradiographs of 6 μm sections of sycamore cells after 10 weeks on the agar medium of Murashige and Skoog with myo-inositol-2-3H.
alkali.
The presence of free glucuronic acid in the ethanol soluble fraction is presumptive evidence for a pathway of myo-inositol metabolism in which uronic acid is produced by ring cleavage. Some significance attaches to the fact that no randomization of label into hexose or other cell constituents was observed. However, it was previously shown that less secondary redistribution of label into hexose occurred when myo-inositol-2,4,6-H was supplied than when myo-inositol-2-14C was provided (23). In the conversion of myo-inositol-2,4,6-H to pentose, tritium is limited stereochemically to hydrogen in the R position of carbon-5 of pentose. Conversion of this pentose to glucose-6-P will include an exchange with the medium of any tritium that redistributes into carbon-1 (22). Nevertheless, other possibilities exist for the utilization of free pentose and some redistribution of label would be expected either if cell wall polysaccharides broke down extensively during growth or if myo-inositol was directed into pathways analogous to the glu-
curonic acid pathway known in animals (15).

Composition of the Cell Wall. Polysaccharides rich in galacturonic acid are generally regarded as characteristic non-cellulosic carbohydrates of primary cell wall and middle lamella in higher plants. Transition from primary to secondary wall sub-
stance is accompanied by a significant change in composition from galacturonic acid-rich polymers to xylose-rich polymers. Included in this change is appearance of 4-o-methyl glucuronic acid as non-reducing units attached to the xylose chain. Glucuronic acid units are also found, but usually as non-reducing single units attached to xylose, never as homopolysaccharide structures (3, 28).

Conventional procedures for separating cell wall polysaccharides usually begin with a mild extractive procedure: water, ammonium oxalate, or EDTA. This solubilizes pectic substance, the polysaccharide components rich in galacturonic acid, arabinose and galactose. Subsequent steps, usually of a more drastic chemical nature, release xylose-rich polymers and quantities of polysaccharides rich in glucose, mannosé and galactose units. In their study of wood polysaccharides, Thornber and Northcote (31, 32) referred to galacturonic acid-rich polysaccharide or pectic substance as a fraction extracted during 4 hours at room temperature in 0.2 M EDTA after prior treatment of the tissue with water, ethanol and benzene. They referred to xylan-rich polysaccharide or hemicellulose as a fraction recovered from the delignified residue of EDTA-extracted tissue by treatment with strong alkali. The only uronic acid found in pectic sub-
stance was galacturonic acid while 4-o-methyl glu-
curonic acid predominated in hemicellulose.

In our study, a combination of EDTA and pecti-
nase released and hydrolyzed over one-third of the polysaccharides accompanied by an equal propor-
tion of label. Three characteristic monosaccharide constituents of pectic substance, galacturonic acid, galactose and arabinose, accounted for 70% of all carbohydrate released.

Alkali released another considerable fraction of the cell wall residue into solution. Fractionation by procedures normally applicable to hemicellulose (29) produced 3 fractions, one insoluble in neutral aqueous solution, a second insoluble in aqueous ethanol but soluble in water and a third soluble in aqueous ethanol. Superficially, the first 2 fractions resembled hemicellulose A and B in terms of solubility but there the similarity ended. Neither contained glucuronic acid or its 4-o-methyl derivative. Both were very poor in xylose units. Successive fractions, IIIa, b and c, of alkali-extracted carbohydrate became progressively richer in arabinose and progressively poorer in galacturonic acid, the only uronic acid present. Based upon uronic acid and pentose composition, these non-cellulosic polysaccharides were totally pectic.

An alcohol-soluble polysaccharide rich in arabi-
nose was first described by Hirst and Jones (14). It was extracted from arabinan-pectic acid complex of peanut with 70% (v/v) ethanol. The polymer contained 95% arabinose. A similar polysaccharide has been recovered from pectic substance extracted by lime-water from sugar beets by Hullar (16). Apparently such arabinose structures are fragments of more complex pectic structures. Similar arabinose-rich carbohydrates have been isolated from apple pectin (4) and mustard-seed embryos (13). The data obtained in the present study indicates that sycamore cell cultures contain pectic heteropoly-
saccharide in which arabinans are linked through alkali-labile bonds to more complex structures.

It is significant that cambial cells removed from sycamore trees contained both pectic substance and hemicellulose (31, 32), yet the chemical studies here on cell cultures initiated from this type of tissue, produce only carbohydrates with a composition re-
sembling pectin. This suggests that the processes involved in the biosynthesis of secondary cell wall polysaccharides were not activated. No information was acquired, however, about the controls which might be operating on the biosynthetic pathways involved. Such information might be usefully gained by manipulation of the growth medium in such a way as to induce new patterns of cell wall development.

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Literature Cited


