Metabolic Studies on Intermediates in the myo-Inositol Oxidation Pathway in Lilium longiflorum Pollen

I. CONVERSION TO HEXOSES

Received for publication June 28, 1977 and in revised form September 26, 1977

CLAIRE-LISE ROSENFIELD,2 CAREY FANN,3 AND FRANK A. LOEWUS4

Department of Biological Sciences, State University of New York, Buffalo, New York 14214

ABSTRACT

The myo-inositol oxidation pathway was investigated in regard to its role as a source of carbon for products of hexose monophosphate metabolism in germinated pollen of Lilium longiflorum Thunb., cv. Ace. myo-[2-14C]inositol and D-[1-14C]glucose had similar distributions of radioactivity, contributing about three times more label to polysaccharide-bound glucose than myo-[2-3H]inositol. In the presence of glucogenesis label from the latter appeared as tritiated water in the medium. This exchange could be enhanced by supplying D-[5R,5S-3H2]xylose instead of myo-[2-3H]inositol. When the former was administered, [3H]glucose was the only labeled sugar residue found in polysaccharide products. The soluble constituents of D-[5R,5S-3H2]xylose-labeled pollen contained no traces of labeled xylose despite massive uptake and utilization.

L-[1-14C]- and L-[5-14C]-Arabinose produced similar labeling patterns in germinated pollen including incorporation of arabinosyl units into pollen tube polysaccharides and substantial glucogenesis which led to utilization of arabinose for respiration and further incorporation of labeled glucosyl units into pollen tube polysaccharides.

D-[5-3H2]Galacturonate was rapidly taken up by germinated pollen but slowly utilized, without conversion to other sugars, for incorporation into pollen tube polysaccharides. L-[6-14C]Gulonate was not taken up by pollen.

Results strongly support a scheme of conversion from myo-inositol to hexose monophosphate and subsequent products of glucose metabolism that involves the myo-inositol oxidation pathway.

When labeled myo-inositol is administered to germinating lily pollen, label appears rapidly in products of glucuronic acid metabolism, mainly uronic acid and pentose units of tube wall polysaccharides. With time, label also appears in polysaccharide-bound glucose (4). This conversion of myo-inositol to glucose has been noted in other plant tissues including parsley leaves (20), strawberry fruits (20), corn seedlings (9), and lily pistils (12), but processes involved in this conversion have yet to be determined.

Biosynthesis of myo-inositol involves direct cyclization of D-glucose-6-P to L-myoinositol-1-P. Conversion of myo-inositol to glucose by reversal of this reaction would require formation of L-myoinositol-1-P. Formation of myo-inositol-1-P from myo-inositol was found in detached corn root tips by J. Dehuss (see ref. 16) and the kinase for this reaction has been described (7) but conversion of L-myoinositol-1-P to D-glucose-6-P by reversal of myo-inositol-1-P synthase could not be demonstrated (21).

Alternatively, conversion of myo-inositol to glucose could occur after oxidative cleavage of myo-inositol to glucuronate. Indeed, when D-[1-14C]glucurono-6,3-lactone was fed to detached strawberry fruits, significant [14C] was recovered in sucrose and xylose (8). Free D-xylose was readily converted to sucrose in the strawberry (17). Animals also convert myo-inositol to hexose over a pathway known as the glucurono-xylulose cycle (25) which involves reduction of D-glucuronic acid to L-gulonic acid. Although plant tissues also reduced D-glucuronic and its lactone to T-gulonic acid (8, 18, 20), L-gulonic acid merely accumulated. When L-[1-14C]- or L-[6-14C]glulono-1,4-lactone was fed to detached strawberry fruits or bean apices, a portion was hydrolyzed to L-gulonic acid but none of the label was found in polysaccharide fractions (2). It seems unlikely that the glucurono-xylulose cycle plays a role in the conversion of myo-inositol to hexose in plants.

The present paper examines the metabolic fate of specifically labeled carbohydrates peculiar to the myo-inositol oxidation pathway and to D-glucuronic metabolism. Results have been obtained from myo-[2-3H]- and myo-[2-14C]inositol, D-[1-14C]glucuronate, D-[5-3H]galacturonate, L-[1-14C]- and L-[5-14C]-arabinose, and D-[5R,5S-3H2]xylose. The findings suggest an intermediate role for UDP-D-xylose and free D-xylose in the conversion of myo-inositol to hexose by germinated lily pollen.

A preliminary account of portions of this study has appeared (23).

MATERIALS AND METHODS

Pollen. Anthers of Lilium longiflorum Thumb., cv. Ace (Easter lily) were collected in a commercial greenhouse on the day of anthesis. Anthers were dried in an open, well ventilated area and shaken on 20-mesh screen to recover the pollen which was stored at 4 C as described earlier (4). Two batches collected in March, 1972 were used in these experiments. Batch A with 50 ± 4% germination was used for uptake studies and batch B with 75 ± 5% for isolation of labeled products.

Uptake of myo-inositol was delayed by the time required for...
emergence of pollen tubes, a lapse of 2 hr under the conditions used in these studies (4). Three hr after suspension of pollen grains in the growth medium, a uniform population of tubes representing most viable pollen grains was obtained.

Conditions for germination were similar to those used previously (4). In the present study, flasks were agitated on a gyratory shaker at 100 rpm and at 28 C, conditions found to be optimal for germination in 3 hr. Reducing the agitation to 50 rpm lowered germination to 65% of the optimal value. Raising the rate to 150 rpm failed to improve germination and increased the likelihood of damage to pollen tubes. Five-mg batches of pollen were allowed to germinate for 3 hr in 1-ml volumes of pentaerythritol medium (4). At the end of that period, a portion of the medium was replaced with fresh medium containing the radioactive substrate under study. With most labeled compounds examined in this study, uptake was linear for at least 6 hr when substrate was not limiting. Respired CO2 was trapped in a small plastic well containing 0.5 ml of 2 n NaOH (item 88230, Kontes Glass Co., Vineland, N.J.).

Radioactive chemicals. myo-[2-3H]inositol (8.4 Ci/mmol) and myo-[2-3H]inositol (4.8 Ci/mmol) and were obtained from stock solutions used in earlier studies. l-[1-14C]Arabinose (0.26 Ci/mol) and D-[1-14C]glucuronate (0.8 Ci/mol) were purchased from Nuclear Research Chemicals, Inc., Orlando, Fla. Purity was established by paper chromatography in solvent B (arabino- nose) and solvent D (glucuronate).

d-[5,5S-3H]Xylose (1.9 Ci/mol) was prepared chemically by oxidation of 1,2-O-isopropylideneglucuronic acid chemically with NaI04 to 1,2-O-isopropyldene-α-D-xylo-pentodialdofuranose (29). The latter was reduced with NaBH4, a modification of the procedure used by Isbell et al. (10). After removal of the isopropyldiene group, the labeled pentose was purified by paper chromatography in solvent A.

d-[5-3H]Galacturonate (approximately 1.5 Ci/mol) was recovered from tube wall hydrolysate of myo-[2-3H]inositol-labeled lily pollen. When a portion of the labeled arabinose was digested with 1-α-arabinofuranosidase and recrystallized, 99% of the label could be accounted for as α-arabinose. Treatment of this diluted sample with sodium periodate yielded formaldehyde from carbon 5. The dimered-formaldehyde derivative accounted for 99% of the 14C present in arabinose.

l-[5-14C]Arabinose (approximately 2 Ci/mol) was recovered from tube wall hydrolysate of myo-[2-14C]inositol-labeled lily pollen. When a portion of the labeled arabinose was digested with 1-α-arabinofuranosidase and recrystallized, 99% of the label could be accounted for as α-arabinose. Treatment of this diluted sample with sodium periodate yielded formaldehyde from carbon 5. The dimered-formaldehyde derivative accounted for 99% of the 14C present in arabinose.

The 70% ethyl alcohol-insoluble residue was air-dried, hydrolyzed with 2 N trifluoroacetic acid (in time course and substrate concentration studies) or commercial pectinase (in studies to determine the distribution of label among carbohydrate moieties). To separate sugar constituents after treatment with pectinase, hydrolysates were first freed of cationic and anionic constituents by passage through ion exchange resin columns and then applied to paper for chromatography in the designated solvent mixture (4). It should be pointed out here that the commercial pectinase (Pectinol R-10) used in this study contained a large variety of glycosidase and glycanase activities including the capacity to hydrolyze starch.

Chromatography. Separations were performed by descending chromatography on Whatman No. 1 or 3MM paper or by ascending chromatography on thin layer plates coated with cellulose. Solvents employed were: (A) ethyl acetate-pyridine-H2O, 8:2:1, v/v; (B) ethyl acetate-pyridine-H2O, 10:6:5, v/v; (C) ethyl acetate-H2O-acetic acid-formic acid, 18:4:3:1, v/v; (D) pyridine-n-butyl alcohol-H2O-benzene, 5:3:1:1, v/v; and (E) ethyl acetate-dimethylformamide-H2O-acetone, 7:5:1:5:1:1, v/v. Appropriate standards were included in each chromatogram. Detection of reducing and nonreducing sugars followed procedures devised by Trevelyan et al. (26) and Usoskin and Rekhter (27).

Radioactive Measurements. Paper and thin layer chromatograms were scanned for radioactive areas at an efficiency of 20% for 14C and 1% for 3H. [3H]Water was separated from solutes present in spent medium by sublimation. Soluble sugars and related compounds were dissolved in 1-ml volumes of water in 20-ml glass counting vials and analyzed for radioactivity by the addition of 10 ml of toluene-Triton X-100 counting fluid. Water-insoluble samples were suspended in dioxane-naphtha- lene counting fluid containing Cab-O-Sil. Vial samples were counted by liquid scintillation at efficiencies of 66% for 14C and 26% for 3H.

RESULTS

myo-[2-14C]inositol and myo-[2-3H]inositol. At 0.5 mm, this cyclitol was utilized by lily pollen for tube wall pectin biosynthesis at maximal rate (4). In the present experiments, about 56% of myo-[2-14C]inositol present at this concentration was transported into the pollen tubes in 6 hr and 33% appeared in polysaccharides as represented by the 70% ethyl alcohol-insoluble residue (Table I). By contrast, only 44% of the myo-[2-3H]inositol-derived label was recovered from the pollen tissue. Twenty per cent more label was recovered in spent medium after myo-[2-3H]inositol metabolism than after myo-[2-14C]inositol metabolism. This additional label was identified as [3H]water. To test for possible exchange reactions caused by bacterial contamination, some experiments were run in which tetracyclin was present. In absence of borate, a growth requirement for normal pollen tube development was withheld. Results, including controls, are given in Table II. Uptake of myo-[2-3H]inositol from the medium and appearance of 3H as [3H]water in the medium was unaffected by the presence of tetracyclin. Deletion of borate from the medium impaired myo-[2-3H]inositol uptake and greatly reduced the exchange of 3H with the medium. Since traces of boron are stored in the pollen grain, some pollen grains do germinate and produce short tubes. In the present experiment, withholding borate reduced germination about 25% below that observed when borate was added but less than 30% of the germinated grains produced tubes longer than the diameter of the pollen grain. The myo-[2-3H]inositol uptake observed in the absence of added boron is probably a sensitive measure of the boron reserve of pollen grains.

Neutral sugars, recovered by pectinase hydrolysis from the
Table I. Distribution of Radioactivity in Pollen Fractions After Growth in Labeled Substrates

Five mg samples of lily pollen, Batch A, pregerminated in 1 ml of pentaerythritol medium, were given labeled substrate at the concentration listed. Each experiment was run in duplicate. The total radioactivity present in each sample was as follows: [2-14C]inositol, 9.6 μCi; [2-3H]inositol, 2.6; [3H]Water, 0.93; [5-3H]inositol, 0.23; [5-14C]arabinose, 0.14; and D-[5,5-3H]xylose, 5.2.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Labeled compound</th>
<th>Final concn</th>
<th>Time</th>
<th>Soluble fraction</th>
<th>Insoluble fraction</th>
<th>Distribution of radioactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>mM</td>
<td>hr</td>
<td>% of radioactivity</td>
<td>% of radioactivity</td>
<td>supplied to the medium</td>
</tr>
<tr>
<td>[2-14C]Inositol</td>
<td>0.56</td>
<td>6</td>
<td>29</td>
<td>19</td>
<td>9.5</td>
<td>15</td>
</tr>
<tr>
<td>[2-3H]Inositol</td>
<td>0.56</td>
<td>6</td>
<td>49(21)</td>
<td>16</td>
<td>8.8</td>
<td>16</td>
</tr>
<tr>
<td>[1-14C]Gluconate</td>
<td>0.29</td>
<td>6</td>
<td>31</td>
<td>12</td>
<td>8.5</td>
<td>25</td>
</tr>
<tr>
<td>[1-14C]Arabinose</td>
<td>0.53</td>
<td>3</td>
<td>36</td>
<td>22</td>
<td>0.9</td>
<td>10</td>
</tr>
<tr>
<td>[5-14C]Arabinose</td>
<td>0.53</td>
<td>6</td>
<td>36</td>
<td>16</td>
<td>1.0</td>
<td>8</td>
</tr>
<tr>
<td>[5R,5S-3H]Xylose</td>
<td>0.87</td>
<td>6</td>
<td>68(59)</td>
<td>9</td>
<td>1.1</td>
<td>1.1</td>
</tr>
<tr>
<td>[5R,5S-3H]Xylose</td>
<td>0.87</td>
<td>6</td>
<td>68(59)</td>
<td>9</td>
<td>1.1</td>
<td>1.1</td>
</tr>
</tbody>
</table>

\(^a\)Portion of spent medium recovered as [3H] water shown in parentheses.

\(^b\)Refers to 70% ethyl alcohol soluble and insoluble fractions.

Table II. Effect of Borate-Free Medium on Uptake and Exchange of Label in [myo-[2-3H]inositol and D-[5R,5S-3H]xylose Metabolism by Lily Pollen in the Presence and Absence of Tetracyclin.

Duplicate 5 mg samples of lily pollen (Batch A) were germinated for 3 hr in 1-ml volumes of borate-free pentaerythritol medium with or without tetracyclin added as indicated in the table. At 3 hr, 100 μg of [myo-[2-3H]inositol or D-[5R,5S-3H]xylose were added. Labeled samples were incubated for 6 hr ([2-3H]inositol) or 3 hr ([5R,5S-3H]xylose) and then analyzed. In separate assays, the radioactive activity of tetracyclin was checked by spreading aliquots of 4-hr germinated pollen suspensions on sterile nutrient agar. After 2 days at 25°C, samples containing tetracyclin were devoid of bacterial colonies whereas numerous colonies were found in the absence of the antibiotic.

<table>
<thead>
<tr>
<th>Labeled substrate</th>
<th>Tetracyclin</th>
<th>Borate</th>
<th>Pollen [3H] Water in Tissues</th>
<th>Radioactive Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ug/ml</td>
<td>mM</td>
<td>% of radioactivity supplied</td>
<td></td>
</tr>
<tr>
<td>[2-3H]inositol</td>
<td>0.16</td>
<td>0.16</td>
<td>58</td>
<td>18</td>
</tr>
<tr>
<td>[2-3H]inositol</td>
<td>0.16</td>
<td>0.16</td>
<td>57</td>
<td>16</td>
</tr>
<tr>
<td>[2-3H]inositol</td>
<td>0</td>
<td>0</td>
<td>20</td>
<td>2</td>
</tr>
<tr>
<td>[2-3H]inositol</td>
<td>0</td>
<td>5</td>
<td>21</td>
<td>3</td>
</tr>
<tr>
<td>[5R,5S-3H]xylose</td>
<td>0.016</td>
<td>0.016</td>
<td>26</td>
<td>25</td>
</tr>
<tr>
<td>[5R,5S-3H]xylose</td>
<td>0.016</td>
<td>0.016</td>
<td>24</td>
<td>23</td>
</tr>
<tr>
<td>[5R,5S-3H]xylose</td>
<td>0</td>
<td>0</td>
<td>12</td>
<td>4</td>
</tr>
<tr>
<td>[5R,5S-3H]xylose</td>
<td>0</td>
<td>0</td>
<td>11</td>
<td>3</td>
</tr>
</tbody>
</table>

70% ethyl alcohol-insoluble fraction, were separated by paper chromatography in solvent A and scanned for radioactivity (Fig. 1). Areas corresponding to glucose, arabinose, and xylose were labeled in both experiments but significantly less isoep and relative to arabinose, appeared in glucose when myo-2-[3H]inositol was used, about 70% less than that from myo-2-[14C]inositol (Table I).

These experiments confirm previous observations (4, 15, 20) in which myo-inositol-linked glucogenesis was observed in plant tissues. It was the appearance of [3H] water in the medium when myo-2-[3H]inositol was supplied that prompted more detailed studies on the metabolic fate of D-[5-3H]xylose, itself a conspicuous product of myo-inositol metabolism (20).

Fig. 1. Radiocromatographic scans of neutral sugars from pectinase hydrolysates of pollen tubes labeled with myo-[2-3H]inositol and myo-[2-14C]inositol. Hydrolysates were pretreated by passage through ion exchange resins to remove both cationic and anionic constituents.
D-[1-14C]glucuronate by pollen were followed for 6 hr. Similar results were obtained at both concentrations. Those obtained at 0.04 mM glucuronate are shown in Figure 2A. Production of 14CO2 was negligible. Most of the label quickly appeared in 70% ethyl alcohol-insoluble residues in which accumulation proceeded at a linear rate over the entire 6-hr period. At higher concentrations, incorporation into polysaccharides was still rapid but no longer linear (Fig. 2B) although transport into the soluble fraction of the pollen tubes remained linear. Hydrolysis of 70% ethyl alcohol-insoluble residue from pollen labeled with 0.3 mM D-[1-14C]glucuronate for 6 hr gave a distribution pattern quite similar to that obtained with myo-[2-14C]inositol-labeled pollen (Table 1).

D-[5-3H]Galacturonate. Although D-[5-3H]galacturonate was readily taken up by germinating pollen (Fig. 3A), most of it remained in the soluble fraction. Incorporation into pollen tube polysaccharides was very slow, at 6 hr only 4% appeared in the 70% ethyl alcohol-insoluble residue. The time course experiment in Figure 3A was run at 0.5 mM D-[5-3H]galacturonate, the highest value tested in Figure 3B. Presumably, pollen would tolerate a much higher concentration of galacturonate than it had been tested but it is doubtful that incorporation into polysaccharide would have been greatly increased. Exchange of 3H with the medium was negligible at all concentrations tested. Hydrolysis of the polysaccharide fraction with pectinase released most of the 3H as labeled galacturonate.

L-[6-14C]Gulonate. L-Gulonate is an intermediate in the conversion of myo-inositol to hexose in animal tissue (1). When 0.26 mM L-[6-14C]gulonate was supplied to germinated lily pollen for 6 hr, none of the label was transported into the pollen tubes.

L-[1-14C]Arabinose and L-[5-14C]Arabinose. L-Arabinosyl moieties of pollen tube wall polysaccharides can be derived directly from myo-inositol or D-glucuronate metabolism. Exogonously supplied l-arabinose also furnishes this pentosyl unit to pectic substance. Pollen grown in 0.73 mM L-[1-14C]arabinose removed nearly 70% of the pentose in 6 hr (Fig. 4A). Transport of label into soluble constituents of the pollen tube, incorporation of label into polysaccharides, and release of label as 14CO2 all exhibited linear rates. The departure from this linear rate by the 70% ethyl alcohol-soluble fraction after 4 hr probably reflects depletion of label in the medium. About 50% of the L-[1-14C]arabinose was utilized for polysaccharide biosynthesis. Another 25% was catabolized to CO2. The time course experiment was run at an arabinose concentration that gave maximal incorporation into tube wall arabinosyl units (Table 1). If the arabinose concentration was increased substantially, excess arabinose was drained off through catabolic processes leading to CO2 and hexose formation. Percentage values in Table 1 can be misleading. At 0.53 mM L-[1-14C]arabinose (80 μg/ml), 5 mg of pollen in 1 ml of medium utilized 12 μg of substrate to form arabinosyl units and 15 μg to form glucosyl units of pollen polysaccharides. At 1.53 mM (230 μg/ml), the corresponding values were 17 and 36 μg. Clearly, as the arabinose concentration of the medium rose above 1 mM, direct insertion of arabinose into polysaccharides reached a limit while hexose formation continued to increase.

Results similar to those just described also apply to L-[5-14C]arabinose (Table 1). This is also seen in radiochromatogram scans of neutral sugars from hydrolysates of pollen tube polysaccharides (Fig. 5). Over 90% of the label was located in glucose and arabinose.
Fig. 4. Uptake and utilization of L-[1-14C]arabinose as a function of time (A) and concentration (B). Symbols are the same as in Figure 2. Total 14C in all three fractions is also given (■ ■ ■). Data in plot B were gathered after 3 hr of labeling.

Fig. 5. Radiochromatographic scans of neutral sugars from pectinase hydrolysates of pollen tubes labeled with L-[1-14C]arabinose and L-[5-14C]arabinose. Hydrolysates were pretreated by passage through ion exchange resins to remove both cationic and anionic constituents. Data are shown for pollen samples that were grown in labeled medium for 3 hr (— — —) and 6 hr (—— —).

D-[5R,5S-3H]Xylose. The most distinguishing feature of D-[5R,5S-3H]xylose metabolism in germinated lily pollen was the large amount of 3H released as [3H]water (Fig. 6A). After a brief lag period, 3H exchanged with the medium at a linear rate. Data were gathered for Figure 6A at 0.67 mm D-xylose, well below the point at which production of [3H]water and incorporation of label into polysaccharide became maximal (Fig. 6B). These processes did not saturate until about 3 to 4 mm D-xylose.

As in the case of myo-2-3H]inositol metabolism, [3H]water was produced from D-[5R,5S-3H]xylose by growing pollen tubes, not by contaminating bacteria. Addition of tetracyclin to inhibit bacterial growth had no effect on [3H]water production by D-[5R,5S-3H]xylose-labeled pollen (Table II). Withholding borate from D-[5R,5S-3H]xylose-labeled growth medium to limit pollen tube formation reduced production of [3H]water by 85% (Table II).

The possibility that growing pollen tubes secrete enzymes or discharge cellular contents into the medium thus exposing D-[5R,5S-3H]xylose to reactions involving 3H exchange was also considered. A suspension of pollen that had been pregerminated for 3 or 5 hr was filtered through Miracloth to remove pollen tissues. D-[5R,5S-3H]Xylose was added to the filtrate and allowed to incubate for 2 hr. At the end of this period, no [3H]water was found in the medium.

Distributions of 3H in [3H]water and pollen fractions after growth in 0.87 and 3.53 mm D-[5R,5S-3H]xylose (130 and 530 μg/ml) are listed in Table I. At the higher level, 49% of the 3H exchanged with water in 6 hr. Another 23% remained in the polysaccharide fraction as glucose (see following paper). To make sure that 3H in the sublimates from the spent medium was [3H]water, the sublimates were diluted 100-fold with water and redistilled at atmospheric pressure. A fraction removed after establishing reflux conditions had 95% of the specific activity of the sublimates. Identity of labeled constituents in the soluble fraction of the pollen was not closely investigated. After chromatography on the polysaccharides, chromatography revealed glucose to be the major labeled sugar (Fig. 7). Again, no 3H was found in the xylose region. At least 80% of the 3H in the hydrolysate chromatographed as glucose.

DISCUSSION

These results are consistent with the idea that conversion of myo-inositol to hexose in germinated lily pollen involves the myo-inositol oxidation pathway. The specific steps are outlined in the right hand portion of Figure 8. The first step, oxidative cleavage of myo-inositol to d-glucurionate, is deduced from results in this and earlier studies on over-all conversion of myo-inositol to galacturonic acid and arabinosyl units of pectin. The enzyme catalyzing this reaction, myo-inositol oxygenase, has been purified from oat seedlings (11) but not from pollen. Its presence in lily pollen is inferred from the observation that glucurionate has been identified as the product of myo-inositol metabolism in that tissue (D. B. Dickinson, personal communication). The second step, phosphorylation of d-glucurionate, is catalyzed by a highly specific kinase. Even d-galacturonate is not a substrate of the lily pollen glucuronokinase (14). The next step, conversion of d-glucurionate-1-P to UDP-d-glucurionate, is catalyzed by an enzyme which has been isolated recently from lily pollen (6). The enzyme that converts UDP-d-glucurionate to UDP-α-xylose, UDP-d-glucurionate carboxy-lyase, has been purified from wheat germ but not from lily pollen. The stereochemical retention of 3H when myo-2-3H]inositol is converted to D-[5-3H]xylose (15) as shown in the following paper may be regarded as indirect evidence for the presence of the carboxy-lyase in lily pollen.
Conversion of UDP-β-xylose to free β-xylose is implied in these studies but the mechanism is obscure. One possibility is stepwise loss of UMP followed by Pi similar to the mechanism proposed for formation of free D-glucurionate from UDP-D-glucuronate in animals (24). There is abundant nonspecific phosphodiesterase in germinated lily pollen to catalyze the first of these two steps (5). To reach the pentose phosphate pathway, β-xylose must be converted to β-xylulose and phosphorylated to β-xylulose-5-P. Both enzymes have been isolated from a variety of plant sources (22, 30) but their presence in lily pollen must still be established. The same comment applies to the commonly accepted steps of pentose phosphate metabolism leading to hexose monophosphate.

In the over-all conversion of myo-[2-3H]inositol to hexose monophosphate according to the plant scheme in Figure 8 there should be no respiratory loss of 14C until hexose products accumulate and undergo metabolic interconversions leading to loss of carbons 1 and/or 6. The small amount of 14CO2 released in 6 hr (Table I) is in accord with this view. A small 14CO2 release (Table I) is also consistent with the over-all conversion of myo-[2-3H]inositol to hexose monophosphate.

Fig. 6. Uptake and utilization of D-[5R,5S-3H]xylose as a function of time (A) and concentration (B). Symbols are the same as in Figure 3. Total 3H in all three fractions is also given (■—■). Data in plot B were gathered after 3 hr of labeling.

Fig. 7. Radiochromatographic scans of neutral sugars from pectinase hydrolysates of pollen tubes labeled with D-[5R,5S-3H]xylose. Hydrolysates were pretreated by passage through ion exchange resins to remove both cationic and anionic constituents. Data are shown for pollen samples that were grown in labeled medium for 3 hr (■—■) and 6 hr (---).

Fig. 8. Diagram of alternate pathways of myo-inositol metabolism in plants and animals. Compounds used as substrates in the present study have been framed.
to hexas with an accompanying exchange of $^3$H into the medium. In the ripening strawberry, free D-xylose was a prominent product of L-arabinose as well as D-galacturonic metabolism (17, 19). The possibility of oxidation of D-galactarate to galactarate was not tested in lily pollen although this conversion was observed in the strawberry.

The facile conversion of L-arabinose to glucosyl as well as arabinosyl units of pollen tube polysaccharides and the substantial release of $^{14}$CO$_2$ from L-[1-$^{14}$C]- and L-[5-$^{14}$C]arabinose-labeled pollen lend support to the view that this pentose contributes to the general carbon requirements of the growing pollen tube. Under normal conditions of pollination, lily pollen tubes penetrate a pistil-secreted polysaccharide exudate rich in arabinogalactans that is used to supply the carbohydrate requirements of new tube formation (13).

When the arabinose concentration of the medium was raised above that level needed to maintain maximal incorporation of arabinosyl units into tube wall polysaccharides, its contribution to hexas products and to respiration increased presumably via epimerization to UDP-xylose and release of free D-xylose (23).

Unlike myo-inositol, D-glucuronate, and L-arabinose, exogenously administered D-xylose enters the scheme (Fig. 8) beyond UDP-D-xylose. Plants lack the capacity to phosphorylate D-xylose and convert it to UDP-D-xylose directly. Results indicate that in germinated lily pollen, conversion to hexas monophosphate is the major metabolic route, accounting for 75 to 80% of the D-xylose present in the medium if one includes exchange of $^3$H from D-[5R,5S-$^3$H]xylose to water as a measure of this conversion along with glucose labeling. Inclusion of the exchange reaction is quite valid in this assessment. Exchange occurs hexas monophosphate formation, glycolysis, and respiration. A further discussion of the process will be reserved for the following paper.

Unlike L-arabinose, D-xylose failed to incorporate directly into pollen tube polysaccharides. Its exclusive appearance in glucosyl residues when supplied as D-[5R,5S-$^3$H]xylose to germinated pollen provided convincing proof of a metabolic role other than direct entry into sugar nucleotide as was the case with L-arabinose. Further evidence was seen in the appearance of $^3$H in galactosyl residues of tube wall polysaccharides. The label in this sugar could only appear after $^3$H reached UDP-D-glucose and the latter epimerized.

Although results obtained with D-galacturonate (19) and L-arabinose (17) are consistent with the plant-mediated pathway from myo-inositol and D-glucuronate to free D-xylose, this pathway does not represent the only possible route to free D-xylose. Theoretically, this pentose may be formed from D-xylose as an end product of reactions proceeding from D-galacturonic acid (Fig. 8). The latter is found among products of both myo-inositol and D-glucuronol-6,3-lactone metabolism in plants (18-20). When L-[1-$^{14}$C]- or L-[6-$^{14}$C]galacturonol-1,4-lactone was administered to detached bean apices or ripening strawberry fruits, a portion was hydrolyzed. This galacturonol portion accumulated in the tissues and was not utilized. None of the $^{14}$C was incorporated into polysaccharides and none was converted to free D-xylose (2). Similar results were obtained when L-[1-$^{14}$C]galacturonol-1,4-lactone was administered to detached spinach seedlings or Oxalis leaves (J. C. Yang and F. A. Loewus, unpublished studies).

The animal-mediated pathway also includes xyitol as an intermediate (25). A report on the occurrence of xyitol in plant tissues has appeared (28). These authors report as much as 935 mg of xyitol/100 g (dry wt) in Italian plum fruits and 258 mg in endive leaves. An attempt to confirm the presence of xyitol in these tissues in this laboratory was unsuccessful (M. W. Loewus, unpublished observation). The Austrian workers used bakers' yeast to remove fermentable sugars. The possibility exists that this treatment may have failed to remove D-xylose and merely reduced it to xylitol (3). Our attempt to confirm Washiuft's finding avoided the yeast fermentation step by using a base-catalyzed oxidation of interfering sugars with Ba(OH)$_2$, prior to gas chromatographic analysis for xylitol. Until a detailed study of xyitol metabolism in plants appears, its role in the conversion of D-glucuronic acid to D-xylose in plants must be regarded with caution.

Acknowledgments — The technical assistance of D. Bedgar in portions of this research is gratefully acknowledged. We also wish to thank D. Dickinson for many thoughtful discussions. Conditions for optimal germination of pollen were studied by H. J. Swartz as part of an undergraduate project.

LITERATURE CITED


16. Loewus F 1969 Metabolism of inositol in higher plants. Ann NY Acad Sci 165: 577-598

17. Loewus FA, R Jang 1958 The conversion of C-labeled sugars to D-ascorbic acid in strawberries. III. Labeling patterns from benidrines administered D-1-$^{14}$C. J Biol Chem 222: 521-532

18. Loewus FA, S Kelly 1959 The conversion of D-glucuronolactone to D-gulonic acid by the detached ripening strawberry. Biochim Biophys Res Commun 1: 143-146


