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

I. CONVERSION TO HEXOSES[1]

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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-^{14}]Inositol and D-[1-^{14}C]glucuronate had similar distributions of radioactivity, contributing about three times more label to polysaccharide-bound glucose than *myo*-[2-^{3}H]inositol. In the course of glucogenesis label from the latter appeared as tritiated water in the medium. This exchange could be enhanced by supplying D-[5R,5S-^{3}H]xylose instead of *myo*-[2-^{3}H]inositol. When the former was administered, [^{3}H]glucose was the only labeled sugar residue found in polysaccharide products. The soluble constituents of D-[5R,5S-^{3}H]xylose-labeled pollen contained no traces of labeled xylose despite massive uptake and utilization.

L-[1-^{14}C]- and L-[5-^{14}C]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-^{3}H]Galacturonate was rapidly taken up by germinated pollen but slowly utilized, without conversion to other sugars, for incorporation into pollen tube polysaccharides. L-[6-^{14}C]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-*myo*-inositol-1-P. Conversion of *myo*-inositol to glucose by reversal of this reaction would require formation of L-*myo*-inositol-1-P. Formation of *myo*-inositol-1-P from *myo*-inositol was found in detached corn root tips by J. Dehusses (see ref. 16) and the kinase for this reaction has been described (7) but conversion of L-*myo*-inositol-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-^{14}C]glucurono-6,3-lactone was fed to detached strawberry fruits, significant ^{14}C 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 glucuronate-xylulose cycle (25) which involves reduction of D-glucuronic acid to L-gulonic acid. Although plant tissues also reduced D-glucuronate and its lactone to L-gulonic acid (8, 18, 20), L-gulonic acid merely accumulated. When L-[1-^{14}C]- or L-[6-^{14}C]gulono-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 glucuronate-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-glucuronate metabolism. Results have been obtained from *myo*-[2-^{3}H]- and *myo*-[2-^{14}C]inositol, D-[1-^{14}C]glucuronate, D-[5-^{3}H]galacturonate, L-[1-^{14}C]- and L-[5-^{14}C]arabinose, and D-[5R,5S-^{3}H]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

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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 gyrorotary 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 CO_2 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-^{14}C]Inositol (11.8 Ci/mol) and *myo*-[2-^{3}H]inositol (4.8 Ci/mol) were obtained from stock solutions used in earlier studies. L-[1-^{14}C]Arabinose (0.26 Ci/mol) and D-[1-^{14}C]glucuronate (0.8 Ci/mol) were purchased from Nuclear Research Chemicals, Inc., Orlando, Fla. Purity was established by paper chromatography in solvent B (arabinose) and solvent D (glucuronate).

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

D-[5R,5S-^{3}H]Xylose (1.9 Ci/mol) was prepared chemically by oxidation of 1,2-*O*-isopropylidene-α-D-glucofuranose with $NaIO_4$ to 1,2-*O*-isopropylidene-α-D-*xylo*-pentodialdofuranose (29). The latter was reduced with NaB^3H_4, a modification of the procedure used by Isbell *et al.* (10). After removal of the isopropylidene group, the labeled pentose was purified by paper chromatography in solvent A.

D-[5-^{3}H]Galacturonate (approximately 1.5 Ci/mol) was recovered from tube wall hydrolysate of *myo*-[2-^{3}H]inositol-labeled lily pollen. Fifty mg of pollen was germinated for 3 hr in 10 ml of pentaerythritol medium (4). At that point, 1.5 mg of *myo*-[2-^{3}H]inositol (26.4 Ci/mol) was added and pollen tube growth allowed to continue for an additional 8 hr. The 70% ethanol-insoluble residues were digested with pectinase (Pectinol R-10, Rohm and Haas, Philadelphia) releasing 35% of the supplied label as soluble products. Ion exchange chromatography yielded a peak of galacturonic acid accounting for 22% of the soluble ^{3}H (8% of ^{3}H supplied). Purity of the galacturonate was confirmed by paper chromatography in solvent C.

L-[6-^{14}C]Gulonic acid was prepared from D-[1-^{14}C]glucuronate by reduction with $NaBH_4$ (2). Purity was confirmed by paper chromatography in solvent E.

Recovery of Labeled Products from Pollen. Labeled tissue was recovered from spent medium by centrifugation at 1,000*g* for 4 min and washed twice with fresh pentaerythritol medium to remove traces of radioactivity from outer surfaces of the intact pollen. Washed pollen was resuspended in 4 ml of 70% ethyl alcohol at 0 C and ground in a glass homogenizer. The suspension was centrifuged at 6,000*g* for 10 min to pellet ethyl alcohol-insoluble residue. The supernatant fluid was removed and the pellet washed with three successive 1-ml portions of 70% ethyl alcohol, lowering the final extractable radioactivity to <1% of that in the initial extract. Extract and washes were combined and assayed for radioactivity.

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-H_2O, 8:2:1, v/v; (B) ethyl acetate-pyridine-H_2O, 10:6:5, v/v; (C) ethyl acetate-H_2O-acetic acid-formic acid, 18:4:3:1, v/v; (D) pyridine-*n*-butyl alcohol-H_2O-benzene, 5:5:3:1, v/v; and (E) ethyl acetate-pyridine-H_2O-acetic acid-methyl alcohol, 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 Usov and Rekhter (27).

Radioactive Measurements. Paper and thin layer chromatograms were scanned for radioactive areas at an efficiency of 20% for ^{14}C and 1% for ^{3}H. [^{3}H]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-naphthalene counting fluid containing Cab-O-Sil. Vial samples were counted by liquid scintillation at efficiencies of 66% for ^{14}C and 26% for ^{3}H.

RESULTS

***myo*-[2-^{14}C]Inositol and *myo*-[2-^{3}H]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-^{14}C]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-^{3}H]inositol-derived label was recovered from the pollen tissue. Twenty per cent more label was recovered in spent medium after *myo*-[2-^{3}H]inositol metabolism than after *myo*-[2-^{14}C]inositol metabolism. This additional label was identified as [^{3}H]water. To test for possible exchange reactions caused by bacterial contamination, some experiments were run in which tetracyclin was present and others in which borate, a growth requirement for normal pollen tube development, was withheld. Results, including controls, are given in Table II. Uptake of *myo*-[2-^{3}H]inositol from the medium and appearance of ^{3}H as [^{3}H]water in the medium were unaffected by the presence of tetracyclin. Deletion of borate from the medium impaired *myo*-[2-^{3}H]inositol uptake and greatly reduced the exchange of ^{3}H 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-^{3}H]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: *myo*-[2-^{14}C]inositol, 9.6 μCi; *myo*-[2-^{3}H]inositol, 2.6; D-[1-^{14}C]glucuronate, 0.03; L-[1-^{14}C]arabinose, 0.23; L-[5-^{14}C]arabinose, 0.14; and D-[5R,5S-^{3}H]xylose, 5.2.

Substrate			Distribution of radioactivity								
Labeled compound	Final concn	Time	Spent medium[a]	Soluble fraction[b]	Insoluble fraction[b] Uronic acid	Ara	Xyl	Glc	Gal	Total	Respired CO_2
	mM	Hr	% of radioactivity supplied to the medium								
[2-^{14}C]Inositol	0.56	6	29	19	9.5	15	1.0	6.9	0.6	33	4
[2-^{3}H]Inositol	0.56	6	49(21)	16	8.8	16	1.2	2.0	<0.1	28	--
[1-^{14}C]Glucuronate	0.29	6	31	12	8.5	25	1.9	5.9	<0.1	41	8
[1-^{14}C]Arabinose	0.53	3	36	22	0.9	10	0.9	12.4	0.4	25	11
	0.53	6	12	20	1.8	16	1.0	19.7	0.7	39	20
	1.53	3	60	13	0.3	5	0.3	8.6	0.1	14	7
	1.53	6	36	16	1.0	8	0.6	16.2	0.3	26	16
[5-^{14}C]Arabinose	0.53	6	14	21	2.4	20	1.5	14.3	1.1	39	17
	1.53	6	27	22	1.6	9	1.0	18.1	0.5	30	14
[5R,5S-^{3}H]Xylose	0.87	3	69(53)	16	1.0	0.9	<0.1	11.9	1.2	15	--
	0.87	6	68(59)	9	1.1	1.1	<0.1	14.7	1.1	18	--
	3.53	3	60(33)	25	0.6	0.4	<0.1	14.2	0.8	16	--
	3.53	6	60(49)	12	0.8	0.7	<0.1	22.8	0.7	25	--

[a]Portion of spent medium recovered as [^{3}H]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-^{3}H]Inositol and D-[5R,5S-^{3}H]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 borate and/or tetracyclin added as indicated in the table. At 3 hr, 100 μg of *myo*-[2-^{3}H]inositol or D-[5R,5S-^{3}H]xylose were added. Labeled samples were incubated for 6 hr ([2-^{3}H]inositol) or 3 hr ([5R,5S-^{3}H]xylose) and then analyzed. In separate assays, the bacteriostatic 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.

Labeled substrate	Tetracyclin	Borate	Distribution of Radioactivity	
			Pollen Tissues	[^{3}H]Water in medium
	μg/ml	mM	% of radioactivity supplied	
[2-^{3}H]Inositol	0	0.16	58	18
	5	0.16	57	16
	0	0	20	2
	5	0	21	3
[5R,5S-^{3}H]Xylose	0	0.16	26	25
	5	0.16	24	23
	0	0	12	4
	5	0	11	3

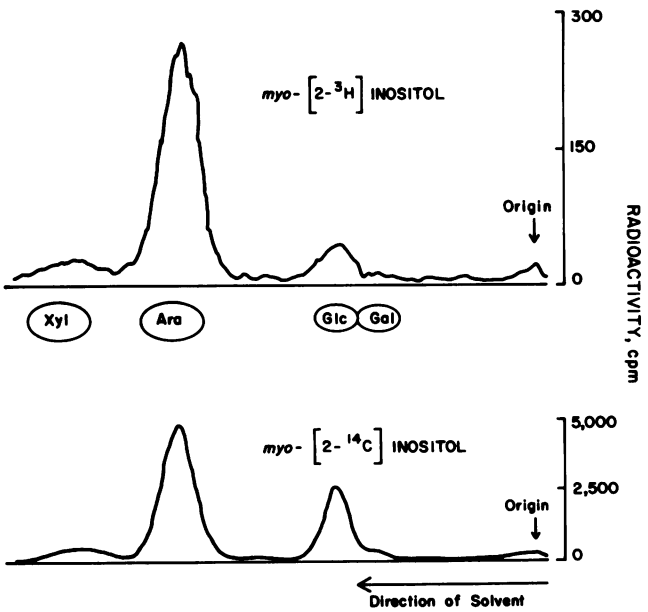

FIG. 1. Radiochromatographic scans of neutral sugars from pectinase hydrolysates of pollen tubes labeled with *myo*-[2-^{3}H]inositol and *myo*-[2-^{14}C]inositol. Hydrolysates were pretreated by passage through ion exchange resins to remove both cationic and anionic constituents.

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 isotope, relative to arabinose, appeared in glucose when *myo*-[2-^{3}H]inositol was used, about 70% less than that from *myo*-[2-^{14}C]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 [^{3}H]water in the medium when *myo*-[2-^{3}H]inositol was supplied that prompted more detailed studies on the metabolic fate of D-[5-^{3}H]xylose, itself a conspicuous product of *myo*-inositol metabolism (20).

D-[1-^{14}C]Glucuronate. D-Glucuronate, product of the oxidative cleavage of *myo*-inositol, is the first step in the conversion of *myo*-inositol to uronic acid and pentose components of pectic substance in plants. Uptake and utilization of 0.04 and 0.3 mM

D-[1-^{14}C]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 $^{14}CO_2$ 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-^{14}C]glucuronate for 6 hr gave a distribution pattern quite similar to the one obtained with *myo*-[2-^{14}C]inositol-labeled pollen (Table I).

D-[5-^{3}H]Galacturonate. Although D-[5-^{3}H]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-^{3}H]galacturonate, the highest value tested in Figure 3B. Presumably, lily pollen would tolerate a much higher concentration of galacturonate had it been tested but it is doubtful that incorporation into polysaccharide would have been greatly increased. Exchange of ^{3}H with the medium was negligible at all concentrations tested. Hydrolysis of the polysaccharide fraction with pectinase released most of the ^{3}H as labeled galacturonate.

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

L-[1-^{14}C]Arabinose and L-[5-^{14}C]Arabinose. L-Arabinosyl moieties of pollen tube wall polysaccharides can be derived directly from *myo*-inositol or D-glucuronate metabolism. Exogenously supplied L-arabinose also furnishes this pentosyl unit to pectic substance. Pollen grown in 0.73 mM L-[1-^{14}C]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 $^{14}CO_2$ 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-^{14}C]arabinose was utilized for polysaccharide biosynthesis. Another 25% was catabolized to CO_2. The time course experiment was run at an arabinose concentration that gave maximal incorporation into tube wall arabinosyl units (Table I). If the arabinose concentration was increased substantially, excess arabinose was drained off through catabolic processes leading to CO_2 and hexose formation. Percentage values in Table I can be misleading. At 0.53 mM L-[1-^{14}C]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-^{14}C]arabinose (Table I). 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.

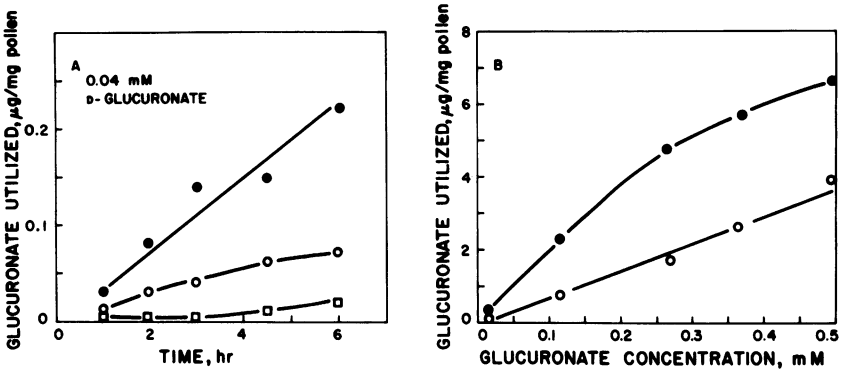

FIG. 2. Uptake and utilization of D-[1-^{14}C]glucuronate as a function of time (A) and concentration (B). Symbols indicate $^{14}CO_2$ released (□——□), ^{14}C taken up and recovered in the 70% ethyl alcohol-soluble fraction (○——○), and ^{14}C incorporated into 70% ethyl alcohol-insoluble fraction (●——●).

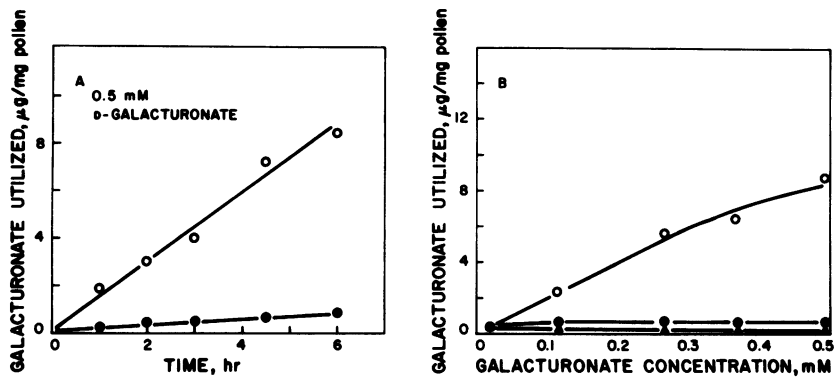

FIG. 3. Uptake and utilization of D-[5-^{3}H]galacturonate as a function of time (A) and concentration (B). Symbols indicate [^{3}H]water in the medium (Δ——Δ), ^{3}H taken up and recovered in the 70% ethyl alcohol-soluble fraction (○——○), and ^{3}H incorporated into the 70% ethyl alcohol-insoluble fraction (●——●).

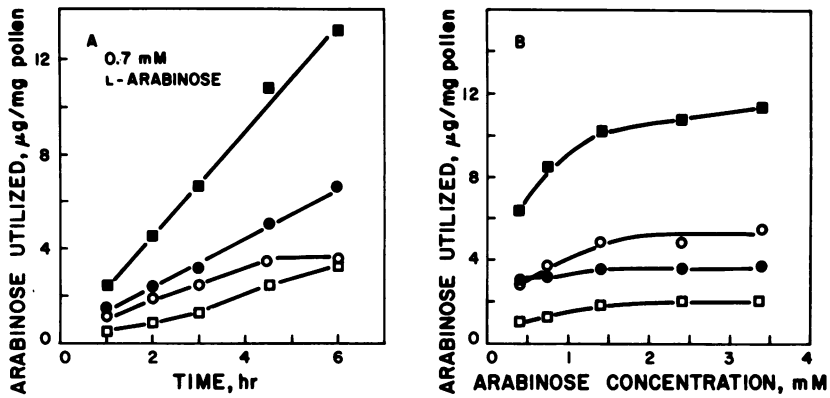

FIG. 4. Uptake and utilization of L-[1-^{14}C]arabinose as a function of time (A) and concentration (B). Symbols are the same as in Figure 2. Total ^{14}C in all three fractions is also given (■——■). Data in plot B were gathered after 3 hr of labeling.

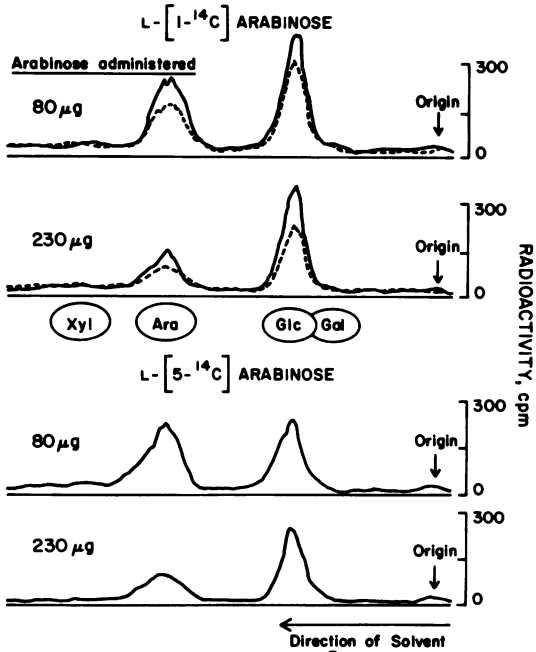

FIG. 5. Radiochromatographic scans of neutral sugars from pectinase hydrolysates of pollen tubes labeled with L-[1-^{14}C]arabinose and L-[5-^{14}C]arabinose. Hydrolysates were pretreated by passage through ion exchange resins to remove both cationic and anionic constituents. Data are shown for pollen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

D-[5R,5S-^{3}H]Xylose. The most distinguishing feature of D-[5R,5S-^{3}H]xylose metabolism in germinated lily pollen was the large amount of ^{3}H released as [^{3}H]water (Fig. 6A). After a brief lag period, ^{3}H 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 [^{3}H]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-^{3}H]inositol metabolism, [^{3}H]water was produced from D-[5R,5S-^{3}H]xylose by growing pollen tubes, not by contaminating bacteria. Addition of tetracyclin to inhibit bacterial growth had no effect on [^{3}H]water production by D-[5R,5S-^{3}H]xylose-labeled pollen (Table II). Withholding borate from D-[5R,5S-^{3}H]xylose-labeled growth medium to limit pollen tube formation reduced production of [^{3}H]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-^{3}H]xylose to reactions involving ^{3}H 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-^{3}H]Xylose was added to the filtrate and allowed to incubate for 2 hr. At the end of this period, no [^{3}H]water was found in the medium.

Distributions of ^{3}H in [^{3}H]water and pollen fractions after growth in 0.87 and 3.53 mM D-[5R,5S-^{3}H]xylose (130 and 530 μg/ml) are listed in Table I. At the higher level, 49% of the ^{3}H exchanged with water in 6 hr. Another 23% remained in the polysaccharide fraction as glucose (see following paper). To make sure that ^{3}H in the sublimate from the spent medium was [^{3}H]water, the sublimate was 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 sublimate. Identity of labeled constituents in the soluble fraction of the pollen was not closely investigated. After chromatography in solvent A, a large radioactive peak remained at the origin. Significant amounts of label were also detected in sucrose, glucose, and fructose. There was no ^{3}H in the xylose region. After enzymic hydrolysis of the polysaccharides, chromatography revealed glucose to be the major labeled sugar (Fig. 7). Again, no ^{3}H was found in the xylose region. At least 80% of the ^{3}H 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-glucuronate, is deduced from results in this and earlier studies on over-all conversion of *myo*-inositol to galacturonosyl 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 glucuronate has been identified as the product of *myo*-inositol metabolism in that tissue (D. B. Dickinson, personal communication). The second step, phosphorylation of D-glucuronate, 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-glucuronate-1-P to UDP-D-glucuronate, is catalyzed by an enzyme which has been isolated recently from lily pollen (6). The enzyme that converts UDP-D-glucuronate to UDP-D-xylose, UDP-D-glucuronate carboxy-lyase, has been purified from wheat germ but not from lily pollen. The stereochemical retention of ^{3}H when *myo*-[2-^{3}H]inositol is converted to D-[5-^{3}H]xylose (15) as shown in the following paper may be regarded as indirect evidence for the presence of the carboxy-lyase in lily pollen.

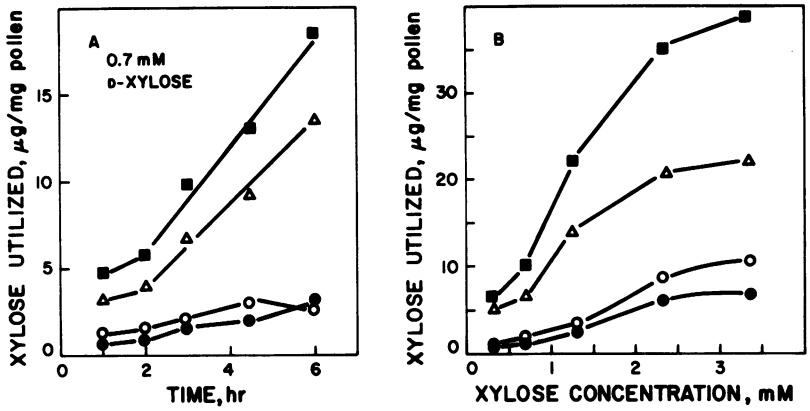

FIG. 6. Uptake and utilization of D-[5R,5S-^{3}H]xylose as a function of time (A) and concentration (B). Symbols are the same as in Figure 3. Total ^{3}H in all three fractions is also given (■——■). Data in plot B were gathered after 3 hr of labeling.

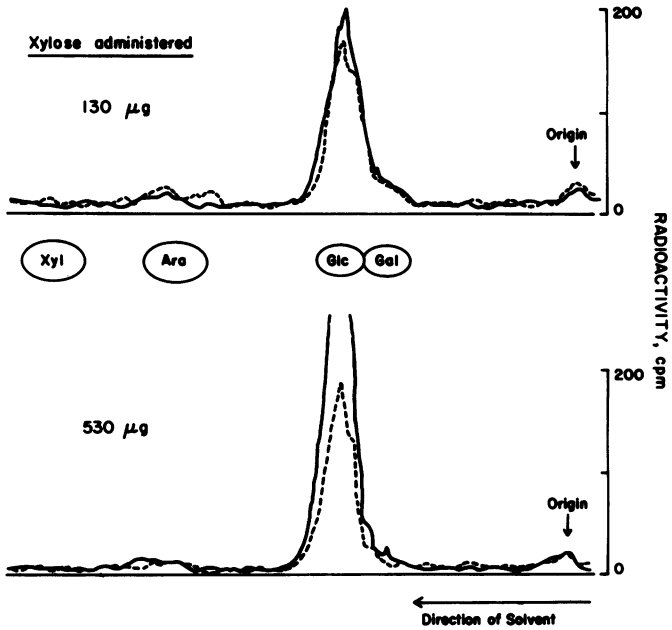

FIG. 7. Radiochromatographic scans of neutral sugars from pectinase hydrolysates of pollen tubes labeled with D-[5R,5S-^{3}H]xylose. Hydrolysates were pretreated by passage through ion exchange resins to remove both cationic and anionic constituents. Data are shown for pollen samples that were grown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

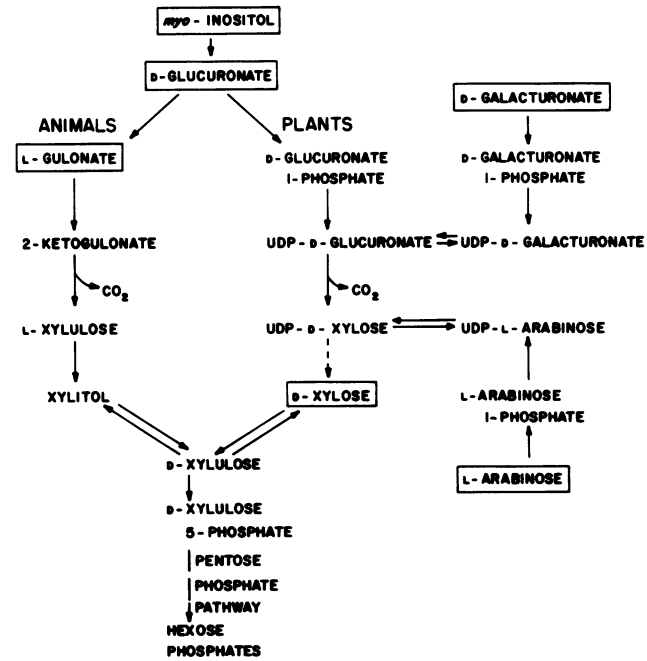

FIG. 8. Diagram of alternate pathways of *myo*-inositol metabolism in plants and animals. Compounds used as substrates in the present study have been framed.

Conversion of UDP-D-xylose to free D-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-glucuronate 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, D-xylose must be converted to D-xylulose and phosphorylated to D-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-^{14}C]inositol to hexose monophosphate according to the plant scheme in Figure 8 there should be no respiratory loss of ^{14}C until hexose products accumulate and undergo metabolic interconversions leading to loss of carbons 1 and/or 6. The small amount of $^{14}CO_2$ released in 6 hr (Table I) is in accord with this view. By contrast, overall conversion of *myo*-[2-^{3}H]inositol to hexose monophosphate will involve loss of ^{3}H by exchange with the medium at the phosphohexose isomerase level (15). Results support this view.

Distribution of ^{14}C among products of D-[1-^{14}C]glucuronate metabolism was similar to that produced with *myo*-[2-^{14}C]inositol (Table I). Such differences as those in labeled arabinose and respired CO_2 probably reflect a more direct channeling of labeled glucuronate into UDP-glucuronate when compared to *myo*-inositol which fulfills compelling cellular requirements other than oxidation to glucuronate.

Membrane impermeability bars the entry of exogenous D-glucuronate-1-P, UDP-D-glucuronate, and UDP-D-xylose to pollen tubes. Access to the latter intermediate can be obtained by supplying L-arabinose since the appropriate kinase and pyrophosphorylase activities occur in plants (17). Although D-[5-^{3}H]galacturonate was readily taken up by germinated pollen tubes, incorporation into pectic substance was sluggish. Since appearance of [^{3}H]water in the medium was negligible, it was assumed that little if any free D-xylose was formed, as this newly formed D-[5-^{3}H]xylose would have been converted quickly

to hexose with an accompanying exchange of ^3H into the medium. In the ripening strawberry, free D-xylose was a prominent product of L-arabinose as well as D-galacturonate metabolism (17, 19). The possibility of oxidation of D-galacturonate 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 polysaccharidic 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 hexose 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 hexose monophosphate is the major metabolic route, accounting for 75 to 80% of the D-xylose present in the medium if one includes exchange of ^3H 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 during hexose 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 ^3H in galactosyl residues of tube wall polysaccharides. The label in this sugar could only appear after ^3H 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-xylulose as an end product of reactions proceeding from L-gulonate (Fig. 8). The latter is found among products of both *myo*-inositol and D-glucurono-6,3-lactone metabolism in plants (18–20). When L-[1-^{14}C]- or L-[6-^{14}C]gulono-1,4-lactone was administered to detached bean apices or ripening strawberry fruits, a portion was hydrolyzed. This L-gulonate 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]gulono-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 xylitol as an intermediate (25). A report on the occurrence of xylitol in plant tissues has appeared (28). These authors report as much as 935 mg of xylitol/100 g (dry wt) in Italian plum fruits and 258 mg in endive leaves. An attempt to confirm the presence of xylitol 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 Washüttl'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 more detailed study of xylitol metabolism in plants appears, its role in the conversion of D-glucuronic acid to D-xylose in plants must be regarded with caution.

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