Carbohydrate Research, 82 (1980) 333–342

THE C-5 HYDROGEN ISOTOPE-EFFECT IN *myo*-INOSITOL 1-PHOSPHATE SYNTHASE AS EVIDENCE FOR THE *myo*-INOSITOL OXIDATION-PATHWAY*†

MARY W. LOEWUS AND FRANK A. LOEWUS

Department of Agricultural Chemistry, Washington State University, Pullman, Washington 99164 (U.S.A.)

(Received August 10th, 1979; accepted for publication in revised form, December 10th, 1979)

ABSTRACT

The hydrogen isotope-effect that occurs *in vitro* during *myo*-inositol 1-phosphate synthase-catalyzed conversion of D-[5-^{3}H]glucose 6-phosphate into *myo*-[2-^{3}H]inositol 1-phosphate has been used to compare the functional role of the nucleotide sugar oxidation-pathway with that of the *myo*-inositol oxidation-pathway in germinating lily pollen. Results reveal a significant difference between the $^3H/^{14}C$ ratios of glucosyl and galactosyluronic residues from pectinase–amyloglucosidase hydrolyzates of the 70% ethanol-insoluble fraction of D-[5-^{3}H, 1-^{14}C]glucose-labeled, germinating lily pollen. This isotope effect at C-5 of D-glucose that occurred during its conversion into D-galactosyluronic residues of pectic substance is not explained by loss of ^{3}H when UDP-D-[5-^{3}H, 1-^{14}C]glucose is oxidized by UDP-D-glucose dehydrogenase from germinating lily pollen. The evidence obtained from this study favors a functional role for the *myo*-inositol oxidation-pathway during *in vivo* conversion of glucose into galactosyluronic residues of pectin in germinating lily pollen.

INTRODUCTION

Two pathways for conversion of D-glucose into UDP-D-glucuronic acid and products of UDP-D-glucuronic acid metabolism are given in Fig. 1. Evidence for the *myo*-inositol oxidation-pathway has been obtained in several tissues from higher plants, notably detached, corn root-tips[1], germinating lily pollen[2–4], and cultured cells of sycamore-maple[5] and tobacco[6]. A new opportunity to test the role of *myo*-inositol as an intermediate in the biosynthesis of cell-wall galacturonate emerged when it was found that *in vitro* conversion of D-[5-^{3}H]glucose 6-phosphate into *myo*-inositol 1-phosphate by *myo*-inositol 1-phosphate synthase (EC 5.5.1.4) involved

*Dedicated to Professor Stephen J. Angyal on the occasion of his retirement.

†This work was supported by Grant No. GM-22427 from the National Institute of General Medical Sciences, National Institutes of Health, United States Public Health Service, Bethesda, MD 20014. This is Scientific Paper No. 5413, Project 0266, College of Agriculture Research Center, Washington State University, Pullman, WA 99164, U.S.A.

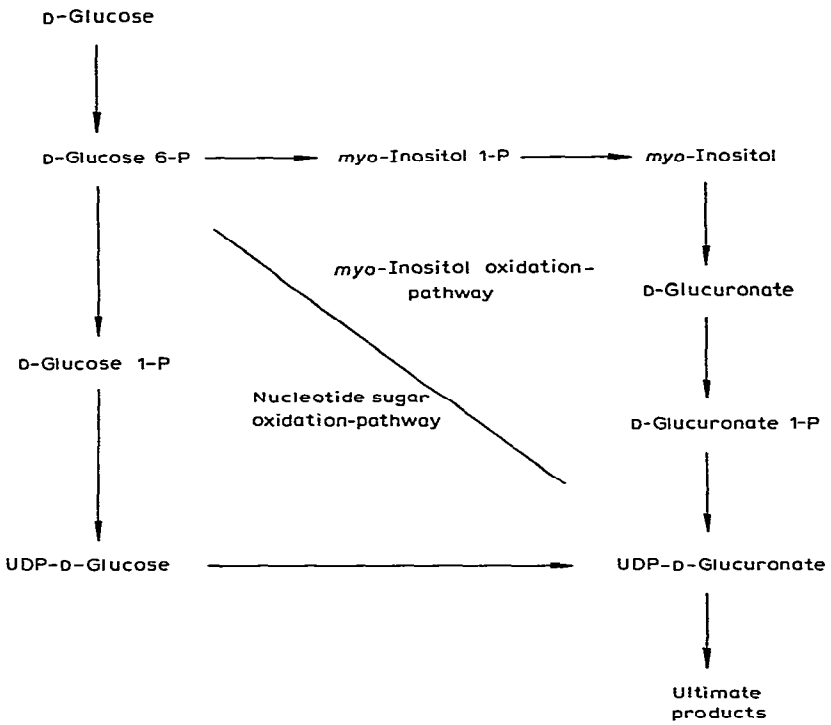

Fig. 1. An abbreviated diagram to show the conversion of D-glucose into UDP-D-glucuronic acid and ultimate products by two separate routes, the *myo*-inositol oxidation-pathway and the nucleotide sugar oxidation-pathway.

a hydrogen isotope-effect[7]. This effect, expressed as a ratio of the velocities $v_{^3H}/v_{^1H}$, varied from 0.21–0.35 for enzyme from rice and sycamore-maple suspension-cell cultures, to 0.48 for freshly prepared, rat testis enzyme[7]. An effect of this magnitude might be reflected in discrimination against 5-tritiated substrate during *in vivo* conversion of D-glucose into D-galactosyluronic residues of tube-wall polysaccharide in germinating lily pollen[8]. The nucleotide sugar oxidation-pathway would not be expected to show this effect. However, Prihar and Feingold[9] recently reported loss of H-5 from UDP-D-glucose during oxidation by UDP-D-glucose dehydrogenase (EC 1.1.1.22) from bovine liver.

The present paper describes experiments in which germinating lily pollen was grown in pentaerythritol media containing D-[5-^{3}H, 1-^{14}C]glucose. Ratios of ^{3}H/^{14}C in glucosyl and galactosyluronic residues of pollen-tube polysaccharides were determined. The ratio obtained from glucosyl residues provided a measure of ^{3}H losses arising from other metabolic processes. UDP-D-Glucose dehydrogenase, isolated from germinating lily pollen, was tested for possible loss of ^{3}H from C-5 during the oxidation of UDP-D-[5-^{3}H, 1-^{14}C]glucose to UDP-D-glucuronic acid.

EXPERIMENTAL

Materials. — Lily pollen (*Lilium longiflorum* Thumb., cv. Ace) was harvested from unopened buds of field-grown 3-year-old plants. Anthers were air-dried for 3 days at 23° and then shaken mechanically in a No. 10 brass sieve to recover the pollen. The pollen was loosely packed in 20-mL polyethylene vials closed with perforated caps and stored at 4°. Alternatively, pollen could be stored at −15° in 50-mL plastic vials fitted with solid closures. Pollen used in these studies was stored for <9 months. D-[5-^{3}H]Glucose (Amersham), D-[1-^{14}C]glucose, and UDP-D-[U-^{14}C]glucose (New England Nuclear) were checked for purity by chromatography and used without further purification. An old sample of D-[1-^{3}H]glucose (Volk) was purified by column chromatography on microcrystalline cellulose with 10:6:5 ethyl acetate–pyridine–water as solvent. UDP-D-[5-^{3}H]Glucose was prepared from D-[5-^{3}H]glucose as described by Wright and Robbins[10], omitting phosphoglycerate and adding phosphoglucomutase to the yeast extract. The yield was 67%.

Labeling procedure. — Pollen (5 mg/mL) was suspended in pentaerythritol medium[11,12]. Pollenkitt, a gummy, orange residue coating the pollen, was removed by stirring the suspension with a glass rod to which the residue adhered. Aliquots (1 mL) of the suspension were transferred to 10-mL Erlenmeyer flasks for germination. Duplicates were run for each time-period, and a third flask provided material for measuring the percentage of germination. Samples were incubated in a gyrotary shaker at 125 r.p.m. for 3 h at 28°. After 3 h, 0.5 mL of medium was removed and replaced by 0.5 mL of medium that contained the amount of labeled glucose specified. Flasks intended for measurement of germination received unlabeled glucose. Flasks were sealed with rubber stoppers and plastic center-wells (Kontes K-882320) in which was placed 0.4 mL of 2M sodium hydroxide to recover respired carbon dioxide. Labeled pollen samples were analyzed after 0.5, 1, 2, and 3 h. Pollen was filtered through glass-fiber disks (Gelman type E) and washed with ice-cold, unlabeled medium.

Fractionation of pollen. — The filtered pollen, together with its glass-fiber filter, was ground in 70% ethanol and fractionated as described earlier[13]. In the present study, digestion of 70% ethanol-insoluble residue by pectinase and amyloglucosidase was combined into a single step.

Distribution of isotopes in glucose. — Glucose was degraded[14] to determine the label in fragments corresponding to C-1 + 2 + 4 + 5 + 6, C-3, C-4 + 5 + 6 and, by difference, C-1 + 2. The ^{14}C in C-1 and C-6 were determined separately by the method of Bloom[15] and ^{14}C in C-4 and C-5 by difference. The ^{3}H at C-2 and C-4 was determined by the change in ^{3}H/^{14}C after oxidation of D-methoxy-D-(hydroxymethyl)diglycolic aldehyde to the corresponding acid[14]. Degradation of D-[5-^{3}H]-glucose with periodate according to Bloom[15] gave incomplete oxidation and ^{3}H appeared in the dimedon derivative, presumably from a 2-carbon aldehyde[16]. The ^{3}H also appeared in the dimedon derivative following periodate oxidation of D-[2-^{3}H]glyceric acid according to Bevill *et al.*[14], indicating that this oxidation is not

reliable when used on certain polyhydroxy compounds in which ^{3}H is attached to the carbon atom adjacent to the group yielding formaldehyde. To obtain unequivocal information regarding ^{3}H attached to C-6, D-glucose was oxidized to D-glucaric acid[17] and the change in $^{3}H/^{14}C$ determined.

Oxidation of UDP-D-[5-^{3}H, 1-^{14}C]glucose and isolation of product. — A preparation of UDP-D-glucose dehydrogenase from germinating lily pollen, taken through the step of ammonium sulfate precipitation[18], was used to convert UDP-D-[5-^{3}H, 1-^{14}C]glucose into UDP-D-glucuronic acid in the presence of a twofold excess of NAD^{+} in 0.1M glycine · HCl buffer, pH 8.7. The reaction was terminated by addition of perchloric acid to 0.55M. The protein precipitated was removed by centrifugation, and perchloric acid, after neutralization with potassium hydroxide, by a second centrifugation. The supernatant solution was passed through a charcoal column (acid-washed Norite A). UDP-D-Glucose and UDP-D-glucuronic acid were eluted together from the column with 1% ammonium hydroxide in 50% ethanol. The eluate was made neutral with formic acid and then dried, redissolved in 0.2 mL of M hydrochloric acid, and placed for 10 min in a boiling-water bath to hydrolyze the nucleotides. Products were separated by paper chromatography and the glucuronic acid further purified by paper electrophoresis.

Analysis of glucose and galacturonic acid. — Glucose was determined by using Worthington's Glucostat (D-glucose oxidase) reagent[19]. Galacturonic acid was determined by the carbazole method[20].

Chromatography and electrophoresis. — Glucose was recovered by descending chromatography in 10:6:5 or 8:2:1 ethyl acetate–pyridine–water. Galacturonic acid and glucuronic acid were separated from other acid components by electrophoresis in 0.1M ammonium formate, pH 3.8, at 20 V/cm for 3 h. Sugars were located by spraying standards on the developed sheets with alkaline silver nitrate. Radioactive components were located with a strip scanner.

Radioactivity. — Dual-labeled samples were analyzed by an external-standard procedure as outlined in the instruction manual of the Packard Model 3320 liquid-scintillation spectrometer. The sodium hydroxide used to trap respired carbon dioxide was diluted tenfold before an aliquot was counted.

RESULTS

Incorporation of ^{14}C from D-[1-^{14}C]glucose into glucosyl and galactosyluronic residues of the pectinase–amyloglucosidase digest (referred to hereafter as the digest) over the period of labeling may be seen in Fig. 2. Some 40% or more of the incorporated ^{14}C entered within 0.5 h after introducing the labeled glucose into the medium. Incorporation into glucan-rich polysaccharide, presumably starch, reached a maximum at 2 h and then declined, even in experiments in which an excess of D-glucose was present in the medium. Galactosyluronic residues did not exhibit this maximum, although the rate of incorporation did diminish with time.

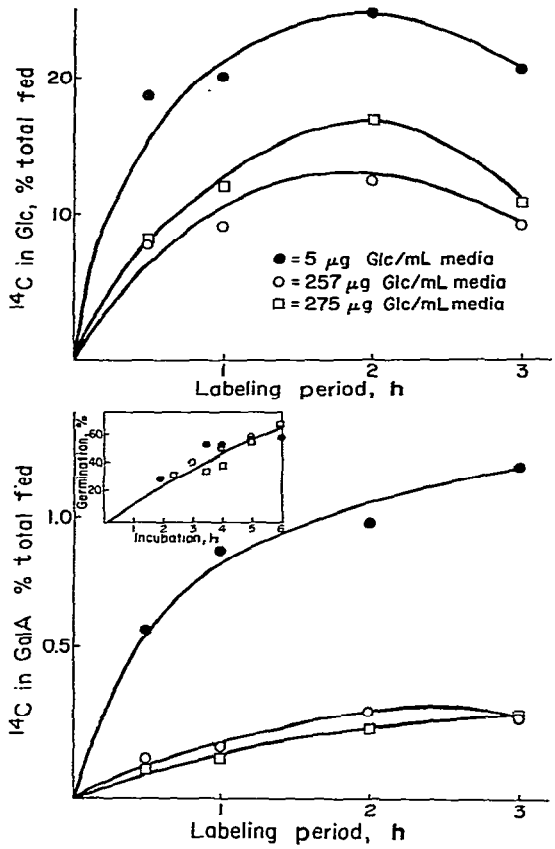

Fig. 2. Incorporation of ^{14}C into glucosyl (upper plot) and galactosyluronic (lower plot) residues from the pectinase–amyloglucosidase-hydrolyzed, 70% ethanol-insoluble fraction of D-[1-^{14}C]glucose-labeled lily pollen. Label was introduced into the growth medium 3 h after germination was initiated (insert).

Germination increased with time, from ~34% at 3 h (the start of labeling) to ~68% at 6 h (Fig. 2, insert).

Table I lists the specific radioactivity of glucosyl and galacturonic acid residues in the digest at intervals following introduction of labeled D-glucose into the medium. Data revealed a major synthesis of glucose- and galacturonic acid-containing polysaccharides in the first 3.5 h of germination, a 3.7-fold increase in total glucose, and about one-half as much in galacturonic acid. The proportion of glucose present in the digest decreased in subsequent sampling-periods, probably as a result of metabolic demands on starch reserves because of pollen-tube elongation. In contrast, the amount of galacturonic acid showed little change. Specific radioactivities of both components increased steadily during the 3 h of labeling, an indication of continuing biosynthesis of starch and pectin. At the end of the experiment described in Table I, 26 μg of glucose remained in the medium. The $^{3}H/^{14}C$ ratio in this glucose was 3.2, as compared with 4.7 in the glucose initially supplied.

TABLE I

SPECIFIC RADIOACTIVITIES OF GLUCOSYL AND GALACTOSYLURONIC RESIDUES FROM THE 70% ETHANOL-INSOLUBLE FRACTION OF D-[5-^{3}H, 1-^{14}C]GLUCOSE-LABELED LILY POLLEN

Period of incubation (h)	*Period of labeling (h)*	*Glucose content[a,b] (μg)*	*Specific radioactivity of glucose (d.p.m. ^{14}C/μg)*	*Galacturonic acid content[b] (μg)*	*Specific radioactivity of galacturonic acid (d.p.m. ^{14}C/μg)*	*Specific radioactivity ratio (Glc/GalA)*
3.5	0.5	392	780	396	15	52
4.0	1.0	404	1040	396	20	52
5.0	2.0	345	1692	390	35	48
6.0	3.0	234	1839	404	43	43

[a]Initial concentration of glucose in medium (1 mL), 257 μg. [b]Values are based on 5 mg of pollen. Ungerminated pollen contained 106 μg of glucose and 245 μg of galacturonic acid (see Experimental Section for assay conditions).

Ratios of $^3H/^{14}C$ in glucose and galacturonic acid from the digest. — These ratios are listed in Table II for three separate experiments, two in which the source of labeling was D-[5-^{3}H, 1-^{14}C]glucose and one, D-[1-^{3}H, 1-^{14}C]glucose. In the first, D-glucose was supplied at 5 μg/mL, an amount insufficient to meet uptake requirements for more than a few min. The other two experiments involved larger initial concentrations of D-glucose, sufficient to supply tissue requirements for the 3-h period of labeling.

At the low glucose concentration, there was a distinct hydrogen isotope-effect in the conversion of D-[5-^{3}H, 1-^{14}C]glucose into D-galacturonic acid, a difference in the $^3H/^{14}C$ ratios of galacturonic acid from the digest to glucose of 0.59 $\pm$0.03. At the higher concentrations of glucose, D-[5-^{3}H, 1-^{14}C]glucose-labeled pollen gave a value of 0.70 $\pm$0.06, which was significantly lower than the value of 0.89 $\pm$0.03 obtained with D-[1-^{3}H, 1-^{14}C]glucose-labeled tissue.

Glucose from digests of pollen supplied with D-[5-^{3}H, 1-^{14}C]glucose had $^3H/^{14}C$ ratios that were 24–29% of that originally added to the media, implying a loss of 71–76% of ^{3}H during 3 h of labeling. During this same period, only 43% of the ^{3}H from D-(1-^{3}H, 1-^{14}C]glucose was lost.

Distribution of label in digest-derived glucose. — Glucose obtained from the sample labeled for 3 h in the high D-[5-^{3}H, 1-^{14}C]glucose medium was degraded to determine its internal distribution of ^{3}H and ^{14}C. Similarly, a sample of the D-[5-^{3}H, 1-^{14}C]glucose supplied was also degraded. Results are given in Table III. The ^{3}H remaining was all at C-5. Of the ^{14}C, originally at C-1, 18% was found at C-6.

Analysis of D-[1-^{3}H, 1-^{14}C]glucose-labeled samples by Bloom's procedure[15] (data not given in Table III) gave 14% of the ^{14}C and 12% of the ^{3}H at C-6 of digest-derived glucose after 0.5 h of labeling, and 20% of the ^{14}C and 18% of the ^{3}H at that carbon atom after 3 h.

TABLE II

$^3H/^{14}C$ RATIOS IN GLUCOSYL AND GALACTOSYLURONIC RESIDUES FROM THE 70% ETHANOL-INSOLUBLE FRACTION OF D-GLUCOSE-LABELED LILY POLLEN

Initial conc. (μg/mL)	*Period of labeling (h)*	*$^3H/^{14}C$ Ratio*		*Ratio GalA / Ratio Glc*
		Glc	*GalA*	
D-[*5-^3H, 1-^{14}C]Glc, $^3H/^{14}C$ = 16.4*				
5	0.5	4.14	2.34	0.57
	1.0	4.09	2.31	0.56
	2.0	4.04	2.52	0.62
	3.0	3.65	2.10	0.58
D-[*5-^3H, 1-^{14}C]Glc, $^3H/^{14}C$ = 4.68*				
257	0.5	1.39	1.07	0.77
	1.0	1.36	1.00	0.72
	2.0	1.38	0.90	0.65
	3.0	1.33	0.86	0.65
D-[*1-^3H, 1-^{14}C]Glc, $^3H/^{14}C$ = 4.65*				
275	0.5	2.67	2.39	0.89
	1.0	2.69	2.31	0.86
	2.0	2.63	2.42	0.92
	3.0	2.66	2.39	0.90

TABLE III

DISTRIBUTION OF ^3H AND ^{14}C IN GLUCOSE OBTAINED FROM D-[5-^3H, 1-^{14}C]GLUCOSE-LABELED LILY POLLEN[a]

Carbon atom	D-[*5-^3H, 1-^{14}C]glucose (source material)*		*Glucosyl residue from pollen*	
	^3H (%)	*^{14}C (%)*	*^3H (%)*	*^{14}C (%)*
1	0	96	0	82
2	0	n.d.[b]	0	n.d.
3	<1	<1	<1	1
4	0	n.d.	0	n.d.
5	103	0	107	0
6	0	0	0	18

[a]Incubation for 3 h with 257 μg of D-[5-^3H, 1-^{14}C]glucose. [b]n.d., not determined.

Oxidation of UDP-D-[5-^3H, 1-^{14}C]glucose by UDP-D-glucose dehydrogenase of lily pollen. — This experiment was undertaken to determine whether a loss of ^3H from C-5 accompanied the conversion of UDP-D-[5-^3H, 1-^{14}C]glucose into UDP-D-glucuronic acid in the presence of lily-pollen enzyme. At the completion of the

reaction, the $^3H/^{14}C$ ratios of D-glucose and D-glucuronic acid recovered from the mixture after acid hydrolysis were 0.495 $\pm$0.002 and 0.473 $\pm$0.028, respectively, from two determinations.

DISCUSSION

The present study sought evidence for a hydrogen isotope-effect *in vivo* similar to the effect found *in vitro* when D-[5-^{3}H]glucose 6-phosphate is converted into *myo*-[2-^{3}H]inositol by *myo*-inositol 1-phosphate synthase[7]. Such evidence would provide new support for the functional existence of a *myo*-inositol oxidation-pathway between hexose and products of glucuronic acid metabolism, specifically the uronic acid and pentose constituents of cell-wall polysaccharides in higher plants. Comparison of $^3H/^{14}C$ ratios in glucosyl and galactosyluronic residues of lily-pollen digests revealed such an effect. The small difference in $^3H/^{14}C$ ratios obtained with D-[1-^{3}H, 1-^{14}C]glucose-labeled pollen is readily explained by partial equilibration of labeled hexose with triose phosphate during hexose phosphate metabolism[21], a process which redistributed ^{3}H to C-6. Subsequent oxidation at C-6 resulted in loss of this ^{3}H.

The process used to recover glucosyl and galactosyluronic residues from the 70% ethanol-insoluble residue of germinating lily pollen did not discriminate among polysaccharidic substrates, but it is safe to assume that starch and the tube-wall pectic substances were the principal sources of these two residues[8,11,22–24].

The figure of 18% for redistribution of ^{14}C from C-1 to C-6 of D-[1-^{14}C]glucose reflects randomization of this label *via* triose phosphate isomerase. Randomization to this extent has been observed to take place in $<$15 min in wheat seedlings, implying at least 36% equilibration of D-glucose 6-phosphate with triose phosphates[21]. An equivalent redistribution of ^{14}C from C-6 to C-1, when D-[6-^{14}C]glucose was fed, was observed by these authors. Tritium at C-5 of D-glucose would be lost during this equilibration. In the experiments described here, 71–76% of the ^{3}H from the fifth position was lost, implying this much equilibration with the triose phosphates. The inequality between randomization of ^{14}C between C-1 and C-6 and the loss of ^{3}H from C-5 might be explained by greater equilibration in the triose phosphate pool of the last three carbon atoms of D-glucose. The 43% loss of ^{3}H from D-[1-^{3}H, 1-^{14}C]glucose during its metabolism by pollen is not explained by the aforementioned process.

Incorporation of radioisotope into galacturonic acid 0.5 h after labeled glucose was added to the medium corresponds to a synthesis of 0.1 pkat per mg of pollen. This figure is calculated from the specific radioactivity of glucose fed, assuming no dilution from existing galactosyluronic residues or precursors. However, such dilution occurs and, when total galactosyluronic residues are determined, their formation during the first 3.5 h of germination represents a synthetis activity of 12.4 pKat per mg of pollen, about 250 times the amount of *myo*-inositol 1-phosphate synthase activity reported for this tissue (0.05 pKat per mg of pollen)[25]. UDP-D-Glucose

dehydrogenase has been reported at an activity of 5.53 pKat per mg of tissue[26]. These observations suggest dominance of the nucleotide sugar oxidation-pathway for biosynthesis of UDP-D-glucuronic acid in germinated pollen, but would be consistent with the *myo*-inositol oxidation-pathway if lily pollen stored large reserves of one or more of the intermediates of that pathway and these intermediates were rapidly converted into galactosyluronic residues during the early stages of germination. This possibility remains to be investigated.

The interesting observation that a portion of H-5 of UDP-D-glucose is lost during the oxidation catalyzed by bovine UDP-D-glucose dehydrogenase[9] could not be repeated with lily pollen as a source of this enzyme. The authors suggest that the loss of tritium at C-5 during this reaction would depend on the dissociation of an aldehyde intermediate from the enzyme, and that the relative affinity of this intermediate may differ in enzymes from different sources. The finding with lily-pollen enzyme appears to rule out an exchange reaction during operation of the nucleotide sugar oxidation-pathway as an explanation for the results obtained in our study.

The isotope effect reported here is consistent with synthesis of galactosyluronic residues *via myo*-inositol 1-phosphate synthase. An untested assumption is made that the glucose 6-phosphate pools used for the synthesis of polymers of D-glucose and D-galacturonic acid are the same, or have undergone identical exchange with triose phosphate pools.

ACKNOWLEDGMENTS

The authors thank N. Yock (Oregon Propagation Co., Brookings) for supplies of lily pollen, and D. Bedgar for technical assistance.

REFERENCES

1 R. M. ROBERTS AND F. LOEWUS, *Plant. Physiol.*, 52 (1973) 646–650.
2 F. A. LOEWUS, M.-S. CHEN, AND M. W. LOEWUS, myo-*Inositol Oxidation Pathway to Cell Wall Polysaccharides*, in F. A. LOEWUS (Ed.), *Biogenesis of Plant Cell Wall Polysaccharides*, Academic Press, New York, 1973, pp. 1–27.
3 I. B. MAITI AND F. A. LOEWUS, *Plant Physiol.*, 62 (1978) 280–283.
4 A. E. MANTHEY AND D. B. DICKINSON, *Plant Physiol.*, 61 (1978) 904–908.
5 R. M. ROBERTS AND F. LOEWUS, *Plant Physiol.*, 41 (1966) 1489–1498.
6 S. HARRAN AND D. B. DICKINSON, *Planta*, 141 (1978) 77–82.
7 M. W. LOEWUS, *J. Biol. Chem.*, 252 (1977) 7221–7223.
8 M.-S. CHEN, M. W. LOEWUS, AND F. A. LOEWUS, *Plant Physiol.*, 59 (1977) 658–663.
9 H. S. PRIHAR AND D. S. FEINGOLD, *FEBS Lett.*, 99 (1979) 106–108.
10 A. WRIGHT AND P. W. ROBBINS, *Biochim. Biophys. Acta*, 104 (1965) 594–596.
11 D. B. DICKINSON, *Plant Physiol.*, 43 (1968) 1–8.
12 D. B. DICKINSON, *J. Am. Soc. Hort. Sci.*, 103 (1978) 413–416.
13 C.-L. ROSENFIELD, C. FANN, AND F. A. LOEWUS, *Plant Physiol.*, 61 (1978) 89–95.
14 R. B. BEVILL, III, E. ALEXANDER, F. SMITH, AND S. KIRKWOOD, *Can. J. Chem.*, 43 (1965) 1577–1587.
15 B. BLOOM, *Anal. Biochem.*, 3 (1962) 85–87.
16 J. GENOVESE, K. SCHMIDT, AND J. KATZ, *Anal. Biochem.*, 34 (1970) 161–169.
17 C. L. MEHLTRETTER, *Methods Carbohydr. Chem.*, 2 (1963) 46–48.
18 M. D. DAVIES AND D. B. DICKINSON, *Arch. Biochem. Biophys.*, 152 (1972) 53–61.

19 R. G. SPIRO, *Methods Enzymol.*, 8 (1966) 2–26.
20 E. A. DAVIDSON, *Methods Enzymol.*, 8 (1966) 52–60.
21 J. EDELMAN, V. GINSBURG, AND W. Z. HASSID, *J. Biol. Chem.*, 213 (1955) 843–854.
22 Y. IWANAMI, *J. Yokohama Municipal Univ.*, Ser. C-34, No. 116 (1959) 1–137.
23 C.-L. ROSENFIELD AND F. A. LOEWUS, *Plant Physiol.*, 61 (1978) 101–103.
24 M.-S. CHEN AND F. A. LOEWUS, *Plant Physiol.*, 59 (1977) 653–657.
25 I. B. MAITI, C.-L. ROSENFIELD, AND F. A. LOEWUS, *Phytochemistry*, 17 (1978) 1185–1186.
26 M. W. LOEWUS AND F. A. LOEWUS, *Plant Physiol.*, 54 (1974) 368–371.