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Metabolism of *myo*-[2-^{3}H]Inositol and *scyllo*-[*R*-^{3}H]Inositol in Ripening Wheat Kernels[1]

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ABSTRACT

Injection of *myo*-[2-^{3}H]inositol or *scyllo*-[*R*-^{3}H]inositol into the peduncular cavity of wheat stalks about 2 to 4 weeks postanthesis led to rapid translocation into the spike and accumulation of label in developing kernels, especially the bran fraction. With *myo*-[2-^{3}H]inositol, about 50 to 60% of the label was incorporated into high molecular weight cell wall substance in the region of the injection. That portion translocated to the kernels was utilized primarily for cell wall polysaccharide formation and phytate biosynthesis. A small amount was recovered as free *myo*-inositol and galactinol. When *scyllo*-[*R*-^{3}H]inositol was supplied, most of the label was translocated into the developing kernels where it accumulated as free *scyllo*-inositol and *O*-α-D-galactopyranosyl-*scyllo*-inositol in approximately equal amount. None of the label from *scyllo*-[*R*-^{3}H]inositol was utilized for either phytate biosynthesis or cell wall polysaccharide formation.

Phytate deposition in wheat, barley and rice accompanies grain development (2, 9). Tanaka *et al.* (21) obtained autoradiographic evidence of this process in wheat and rice by infiltrating [2-^{3}H]MI[3] into the vascular system through a wick that was inserted into the stem of a developing spike. Recently, Ogawa *et al.* (15) obtained labeled phytate from dissected aleurone layers of ripening rice grains that was incubated with ^{32}Pi or [^{14}C]MI. When the same experiment was repeated with [^{14}C]glucose, no incorporation of ^{14}C into phytate occurred. Presumably, the aleurone layer is a major site of phytate biosynthesis in this tissue, but the biosynthetic source of MI exists elsewhere.

Mature grain that contained phytate specifically labeled in the MI portion would provide a useful means of tracing the fate of phytate-derived MI during germination. A previous study involving injection of [2-^{3}H]MI into germinating wheat kernels suggested that phytate-derived MI does contribute to the biosynthetic requirements of the young plant, notably cell wall formation (13). Here, experiments are described in which [2-^{3}H]MI or [*R*-^{3}H]SI[4] was injected into the hollow peduncle of developing wheat spikes, a method successfully used by Sakri and Shannon (17) to prepare labeled wheat kernels. Inclusion of [*R*-^{3}H]SI stemmed from a report (4) that *Klebsiella aerogenes* actively transported MI or SI across the cell membrane but failed to metabolize the latter cyclitol. The results presented here show that, SI, like MI, is transported from site of injection to site of accumulation in the developing kernel but that, unlike MI, SI is not converted to phytate or utilized for cell wall polysaccharide biosynthesis.

MATERIALS AND METHODS

Radiochemicals. *Myo*-inosose-2 was reduced with sodium [^{3}H]borohydride (New England Nuclear Corp.). The products, [2-^{3}H]MI and [*R*-^{3}H]SI, were separated by column chromatography on Whatman CC-31 cellulose (2 × 100 cm) using acetone-H_2O (4:1). Incomplete separation of the two products led to less than 2% cross-contamination, which did not interfere with results obtained. Recently, use of the method described by Spector (20) has provided complete purification of products (N. Tuross and F. A. Loewus, unpublished data).

Plant Material. Wheat was grown in pots under greenhouse conditions or in test plots under field conditions. Six cultivars of *Triticum aestivum* L. (Fielder, Heslop, Stephens, Twin, Wampum, and Yamhill) and one of *Triticum compactum* Host (Barbee) were used. Solutions were injected about 2 cm below the base of the emergent spike (17). A vent about 8 cm below the site of injection facilitated flow of 20 to 100 μl injected solution into the hollow cavity of the peduncle. This vent was made with a fine-bore hypodermic needle which was left in place during the injection.

Labeled spikes were harvested by severing the stem below the uppermost node. Movement of label from [2-^{3}H]MI or [*R*-^{3}H]SI to tissues below this node was negligible. After harvest, the severed portion was divided into four parts: flag leaf, lower peduncle and node, upper peduncle including the site of injection, and spike. In some studies of distribution of radioisotope in the spike, this part was subdivided into spikelets and rachis. In others, it was subdivided into kernels and chaff.

Milled Grain. Samples containing 5 to 10 g were ground with a single pass through a type OM Brebender mill. Passage over 0.5- and 0.125-mm sieves gave three fractions, designated bran, middlings, and flour. Appreciable starchy endosperm remained in attachment to bran fragments. The flour fraction was practically pure endosperm. Milling conditions were such that bran, middlings, and flour were recovered on a weight basis in the ratio 1:2:1.

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[3] Abbreviations: MI, *myo*-inositol; SI, *scyllo*-inositol; DMSO, dimethylsulfoxide; TFAA, trifluoroacetic acid; TMS, trimethylsilyl; MI-Gal, 1L-1-(*O*-α-D-galactopyranosyl)-*myo*-inositol (also galactinol); SI-Gal: *O*-α-D-galactopyranosyl-*scyllo*-inositol; HPLC, high-performance liquid chromatography.

[4] Due to the all *trans* configuration of hydroxyl substituents on the cyclohexane ring of SI, the location of ^{3}H cannot be specified. The symbol *R* has been used to describe the random position of 1 atom ^{3}H per labeled molecule of SI.

Bran Fractionation. Bran was pulverized in a hammer mill for 1 min. One-g samples were extracted with DMSO-H_2O (9:1) at 25 C for 6 h to solubilize starchy endosperm (16, 22). The extracted bran was recovered by centrifugation at 12,000*g* for 10 min and washed successively in H_2O, 95% ethyl alcohol, chloroform-methyl alcohol (1:2), and diethyl ether. Phytate was recovered from washed bran by extraction with 0.2% disodium EDTA at 50 C for 1 h. The insoluble residue was treated with 2 M TFAA at 100 C for 2 h to solubilize a mixture of monomeric and oligomeric fragments of hemicellulose. The final acid-resistant residue was combusted in a biological oxidizer to determine remaining radioactivity.

To recover low mol wt components in the DMSO-extracted fraction, this fraction was diluted with 3 volumes 95% ethyl alcohol. The precipitate, primarily starch and pentosans, was removed by centrifugation and the soluble fraction was evaporated to a small volume for subsequent cellulose column chromatography.

Distribution of ^3H among cellular constituents in the region of the injection site was determined on a batch of 16 cv. Fielder plants that had been given [2-^{3}H]MI. A 2-cm-long section, containing the puncture site, was excised from the peduncle. The total sample (dry weight, 0.18 g, 1.2 μCi) was ground in a mortar and extracted successively with 3 ml 80% ethyl alcohol, four 5-ml portions H_2O, and 3 ml 0.2% disodium EDTA. Alcohol and H_2O extracts were passed through a column of Sephadex G-15 to resolve raffinose, sucrose, and glucose components. The column partially separated MI-Gal from sucrose and MI from glucose.

Chromatography. The alcohol-treated, DMSO extract was loaded on Whatman CC-31 cellulose (1.2 × 23 cm) which had been equilibrated previously with acetone-H_2O (9:1). Elution with acetone-H_2O (4:1) removed inositol and inositol galactoside in that order. Radioactive peaks were purified on Sephadex G-10 (1.2 × 50 cm) using 0.1% acetic acid as solvent. Colored impurities acquired during cellulose column chromatography were removed by this treatment.

MI and SI were characterized as their TMS derivatives by GLC (12). Hydrolyzed products of the inositol galactosides were treated with sodium borohydride and converted to the corresponding acetates. These were separated by GLC on a column (0.64 cm × 1.8 m) of 3% XE-60 on Gas-Chrom Q at 180 C. The inositol galactosides were converted to their TMS derivatives in *N,O*-bistrimethylsilyl-trifluoroacetamide containing 1% trimethylchlorosilane (Pierce Chem. Co., Rockford, Ill.). These derivatives of SI-Gal and MI-Gal were separated with 3% OV-17 on Gas-Chrom Q (0.64 cm × 1.8 m) at 230 C. Their retention times, relative to sucrose, were 2.2 and 2.6, respectively.

Separation of inositol from inositol galactoside, especially in hydrolyzed samples of the latter, was done effectively by HPLC on a μBondapak carbohydrate column (Waters Assoc., Milford, Mass.) using acetonitrile-H_2O, 83:17, at a flow rate of 3 ml/min. Under these conditions, MI or SI appeared at 8 min and their respective galactosides appeared at 16 min. Although the μBondapak carbohydrate column did not resolve SI from MI, this resolution was possible with the Aminex HPX-87 (organic acid) column supplied by Bio-Rad (Richmond, Calif.). With the latter, using 0.1 M formic acid at 0.8 ml/min and 40 C, SI emerged at 7.4 min and MI emerged at 8.0 min.

Phytic acid was recovered from EDTA solubles by separation on Sephadex G-25 (2.2 × 74 cm) using 0.1% acetic acid. Fractions containing phytic acid were identified by hydrolyzing 1-ml aliquots with 0.5 ml 10 N H_2SO_4 at 100 C for 48 h. Hydrolysates were analyzed for MI and Pi according to Isaacks *et al.* (7) and for total sugars with phenolsulfuric acid. Phytic acid was further purified and identified by paper electrophoresis in 100 mM sodium oxalate using ammonium molybdate for detection (14). Paper electrophoresis with 50 mM sodium borate at pH 9.3 (1) was used to separate MI and SI. The detection agent was alkaline silver nitrate.

Assay for Radioactivity. Aqueous samples were counted in 5 ml "tritosol" (5) at 12% efficiency. Insoluble samples were combusted in a biological oxidizer (Packard model 306B) and the water of combustion was trapped in Monophase 40 (Packard Instrument Co., Downers Grove, Ill.) for counting.

SI-Gal. This galactoside was prepared from UDP-D-galactose and SI by the enzymic method of Frydman and Neufeld (6). A trace of [*R*-^{3}H]SI was added to the reaction to facilitate recovery of product. Products were adsorbed on a short column of charcoal (acid-washed Norit A). Unreacted SI was removed with a water wash and SI-Gal was eluted with 10% ethyl alcohol. After removal of solvent, the SI-Gal was dissolved in 1 ml H_2O and purified by HPLC. GLC of the TMS derivative of the purified product gave a single peak ($R_{sucrose}$ = 2.2) corresponding to SI-Gal. Hydrolysis in 2 M TFAA at 100 C for 2 h, treatment with sodium borohydride, acetylation, and separation by GLC revealed two components with retention times corresponding to the hexaacetates of galactitol and SI. Consistent with the finding of Frydman and Neufeld (6), the enzymic product, SI-Gal, was cleaved by α-galactosidase from green coffee beans (Sigma Chem. Co., St. Louis, Mo).

RESULTS

Distribution of Label. In the initial studies with greenhouse-grown plants, about 40 to 50% of the injected label from [2-^{3}H]MI found its way into the spike. Later studies with field-grown plants yielded slightly higher values for translocation. Data in Table I were obtained from greenhouse-grown plants. Here, it was found that virtually all ^3H not translocated to the spike remained at the site of injection. None of the label appeared in the flag leaf and very little below the site of injection. Over 90% of the injected label was recovered. Most of this label was retained by the plant through maturity. Of ^3H translocated to the spike, about 80% appeared in kernels. Caryopses from basal and apical spikelets had higher specific radioactivity than those intermediate. Since

Table I. *Distribution of ^3H in Maturing Tillers of [2-^{3}H]MI- and [R-^{3}H]-SI-injected Wheat*

Tillers were given 20 μl 1.25 mM [2-^{3}H]MI (1 μCi) or 50 μl 0.5 mM (*R*-^{3}H]SI (0.6 μCi) by injection into the peduncular cavity. At intervals, plants were harvested and analyzed for ^3H.

Cultivar	Period of Labeling[a]	Distribution of Radioactivity			
		Lower Peduncle	Upper Peduncle[b]	Flag Leaf[c]	Spike
	days	*% ^3H recovered*			
[2-^{3}H]MI-labeled plants					
Fielder	1	4	56	0	40
	2	4	54	0	42
	7	6	43	0	51
Stephens	7	2	43	0	55
	7	5	54	0	41
	13	2	52	0	46
Heslop	7	6	58	0	36
[*R*-^{3}H]SI-labeled plants					
Twin	18	8	7	0	85
	18	17	8	0	75
	18	18	8	0	74

[a] [2-^{3}H]MI was injected 2 weeks after anthesis and [*R*-^{3}H]SI was injected 4 weeks after anthesis.

[b] This section of the peduncle includes the site of injection.

[c] Less than 0.1%.

the latter region bore spikelets with more kernels and these kernels were, on the average, larger than those at the extremes of the spike, total radioactivity per spikelet was nearly uniform throughout the head. Results similar to that listed in Table I for [2-^{3}H]-MI-labeled plants were obtained for Barbee, Twin, and Yamhill cultivars labeled with [2-^{3}H]MI (data not given).

Plants labeled with [*R*-^{3}H]SI also retained most of the ^{3}H until maturity. Data in Table I were obtained from plants injected with [*R*-^{3}H]SI at 4 weeks postanthesis. Compared to [2-^{3}H]MI-labeled plants, little ^{3}H remained in the vicinity of the site of injection, whereas a somewhat greater amount appeared in the lower portion of the peduncle. More ^{3}H appeared in the spike, amounting to about 75 to 80% of the administered label. In a subsequent experiment involving field-grown plants (cv. Twin) which had been injected with [*R*-^{3}H]SI at 2 weeks postanthesis, 90% of the administered label was recovered from mature kernels.

In the range of 4.5 μg to 4.5 mg, the amount of MI injected per stalk had no measurable effect on the distribution of label from [2-^{3}H]MI in two cultivars, Twin and Wampum.

The stage of maturity selected for injection greatly influenced the amount of MI that reached the spike. About 12 to 16 days after anthesis, translocation was maximal (Fig. 1). Increasing the amount of injected MI from a trace (4.5 μg) to 0.45 mg/stalk had little effect on this maximal value.

Increasing the volume of injected fluid from 10 to 100 μl/stalk produced no measurable differences in translocation of [2-^{3}H]MI over a range of MI concentrations.

Tritium in Peduncular Tissue. Tissue from plants labeled with [2-^{3}H]MI were extracted successively with 80% ethyl alcohol, H_2O, and 0.2% disodium EDTA to remove 17, 34, and 4%, respectively, of the ^{3}H that remained at the site of injection. Gel filtration of the ethyl alcohol and H_2O extracts revealed that less than 14% of the label had the molecular sieving property of MI and that less than 5%, that of MI-Gal. The remaining label in the soluble fraction was associated with oligosaccharides and polysaccharides. Together, soluble and insoluble carbohydrate polymers accounted for about 80% of the ^{3}H found in the region of injection.

Distribution of ^{3}H in Kernels from Wheat Spikes Labeled with [2-^{3}H]MI or [*R*-^{3}H]SI. Pooled samples (five to six spikes) were milled with one pass through the Brebender and sifted into three fractions, bran, middlings, and flour (Table II). Incomplete milling resulted in a bran fraction with about 50% of its dry weight due to endosperm that remained attached to the outer layers of the kernels. Since most of the ^{3}H appeared in the bran and very little in the flour (endosperm), it is reasonable to conclude that the actual specific radioactivity of pure bran was nearly twice as great as the values reported in Table II, whereas relative values reported in Table II for middlings and flour are actually somewhat lower. This pattern of labeling was found in kernels from plants of several cultivars whether the source of label was [2-^{3}H]MI or [*R*-^{3}H]SI. Subsequent experiments involving several hundred field-grown plants confirmed these results and showed that wheat stalks injected with [2-^{3}H]MI or [*R*-^{3}H]SI during the period from 2 to 4 weeks postanthesis translocated the label to the spike where it accumulated in developing kernels, mainly the outer layers (bran fraction), and remained there until harvest.

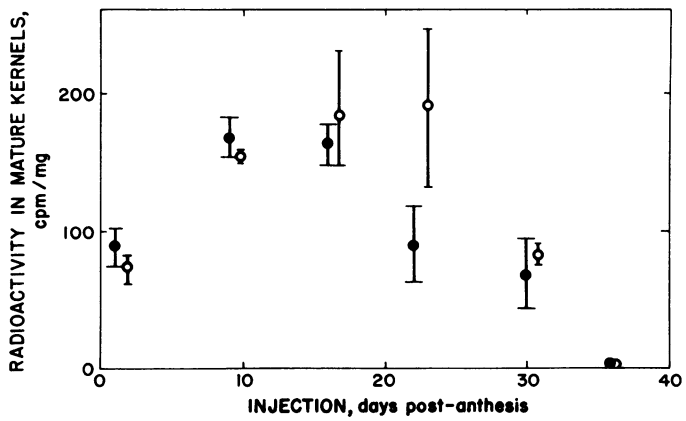

FIG. 1. Appearance of ^{3}H in mature kernels of wheat after injection of [2-^{3}H]MI at increasing stages of maturity. (●), 50 μl 25 μM MI; (○), 50 μl 2.5 mM MI.

An opportunity to compare translocation of [2-^{3}H]MI in uninfected plants with that in plants infected with leaf rust, *Puccinia recondita*, revealed somewhat lower specific radioactivity in kernels from infected wheat but no differences in distribution of ^{3}H among milled fractions (Table II). Mitchell *et al.* (14) found no significant changes in inositol content of wheat leaves infected with stem rust (*Puccinia graminis tritici*) over uninfected controls.

Distribution of ^{3}H in Bran. When kernels were taken from plants labeled with [2-^{3}H]MI, DMSO solubilized only 11% of the incorporated ^{3}H, as compared to 96% when plants were labeled with [*R*-^{3}H]SI (Table III). In both instances, the portion brought into solution, about 60%, was primarily starchy endosperm which had remained in the bran fraction due to incomplete milling. This starch, along with other polysaccharidic substances that had been brought into solution by DMSO (22), could be precipitated with 3 volumes ethyl alcohol, leaving in solution only low mol wt carbohydrates. In the case of [2-^{3}H]MI-labeled bran, the latter accounted for only 4% of the ^{3}H present in crude bran but, in the case of [*R*-^{3}H]SI-labeled bran, 96% remained in solution.

To further characterize the labeled low mol wt fraction from [*R*-^{3}H]SI-labeled bran, this fraction was subjected to cellulose powder chromatography (Fig. 2). Peak I contained a single radioactive compound which was identified as SI by paper electrophoresis and GLC of the TMS derivative. TMS-derivatized peak II gave six components by GLC, only one of which contained significant ^{3}H. This radioactive component had the retention time of SI-Gal. Fractionation of peak II by HPLC gave a single peak in the inositol galactoside region which contained two GLC-detectable components with $R_{sucrose}$ corresponding to SI-Gal and MI-Gal. Over 75% of the ^{3}H was present in SI-Gal. Failure to collect more of the ^{3}H associated with SI-Gal was due to its proximity to MI-Gal present in the bran fraction, which necessitated interruption of the collection of ^{3}H under SI-Gal peak before it had been completely eluted.

Treatment of crude peak II with α-galactosidase led to slow hydrolysis of SI-Gal, releasing 50% of the [^{3}H]SI in 24 h (Table IV). Crude peak II contained about 20 times more MI-Gal than SI-Gal. Slow hydrolysis of SI-Gal in peak II resulted from the presence of MI, a product of MI-Gal hydrolysis. MI is a potent inhibitor of α-galactosidase (19). Under identical conditions, SI-Gal, prepared enzymically according to Frydman and Neufeld (6), was rapidly hydrolyzed by α-galactosidase.

Low mol wt compounds in the DMSO extract of [2-^{3}H]MI-labeled bran represented such a small portion of the total radioactivity in the bran that a separate sample of bran was extracted directly with 80% ethyl alcohol for 3 h at 60 C. Cellulose powder chromatography gave three radioactive peaks (Fig. 3). The first, further separated by Bio-Gel P-2 (Bio-Rad Labs. Richmond, Calif.) gel filtration, gave a single radioactive component which was identified by GLC as MI. The second, also purified on Bio-Gel P-2, gave a single radioactive peak in the same elution volume that characterized sucrose and MI-Gal but which was not identified as either of these compounds. The third, again purified on Bio-Gel P-2, gave a single radioactive peak corresponding to MI-Gal. The unlabeled components included sucrose and raffinose.

Separation of EDTA-soluble compounds from [2-^{3}H]MI-labeled bran on Sephadex G-25 resulted in a large radioactive peak in eluted fractions corresponding to phytic acid (Fig. 4). The latter

Table II. *Distribution of Radioactivity in Milled Fractions*

Cultivar	Relative Radioactivity			Specific Radioactivity[a]		
	Bran	Middlings	Flour	Bran	Middlings	Flour
	%			cpm/mg		
Labeled with [2-^{3}H]MI						
Fielder, rust-free	76	19	5	725 ± 65	90 ± 4	53
Fielder, rust-infected	78	21	1	503 ± 30	77 ± 1	38 ± 3
Twin, rust-free	73	22	5	991 ± 84	158 ± 5	96 ± 2
Twin, rust-infected	72	23	5	738 ± 117	127 ± 4	66 ± 1
Heslop	70	24	5	341 ± 24	61 ± 4	33
Yamhill	72	22	6	381 ± 16	60 ± 5	34 ± 2
Barbee	80	18	2	351 ± 29	60 ± 4	20 ± 1
Labeled with [*R*-^{3}H]SI						
Twin, rust-free	81	18	1	2,811 ± 75	542 ± 5	123 ± 1
Wampum	80	19	1	1,731 ± 142	280 ± 56	86

[a] Duplicate determinations.

Table III. *Distribution of Radioactivity in Bran Fractions*

These samples were obtained from cv. Twin plants which were labeled 2 weeks ([2-^{3}H]MI) or 4 weeks ([*R*-^{3}H]SI) postanthesis and grown to maturity.

Fraction	Radioactivity		Dry Wt	
	[2-^{3}H]MI	[*R*-^{3}H]SI	[2-^{3}H]MI	[*R*-^{3}H]SI
	%		%	
DMSO-soluble	11	96	58	65
EDTA-soluble	41	4	8	6
TFAA-soluble	38	<1	25	24
TFAA-insoluble	5	0	9	5
Recovery	95	100	100	100

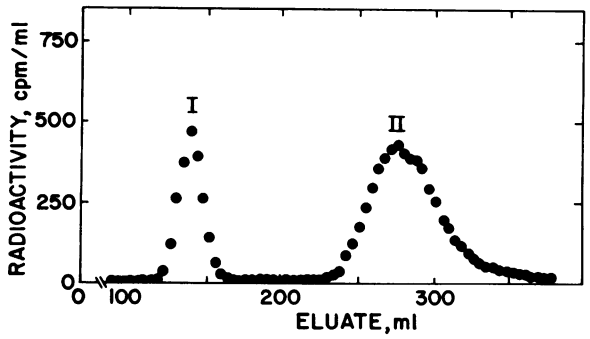

FIG. 2. Cellulose column chromatography of low mol wt compounds from the DMSO extract of [*R*-^{3}H]SI-labeled bran after precipitation of high mol wt polysaccharides with 3 volumes ethyl alcohol.

Table IV. *Hydrolysis with α-Galactosidase of Peak II from Cellulose Column Chromatography*

Hydrolysis	Radioactivity[a]	
	Peak II	SI
h	%	
2	95	5
4	90	10
6	86	14
24	50	50

[a] Substrate and products were separated by HPLC.

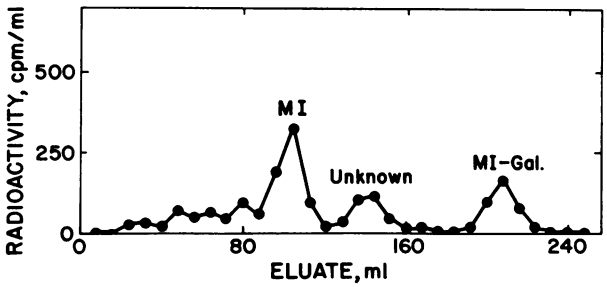

FIG. 3. Cellulose column chromatography of low mol wt compounds that were extracted from [2-^{3}H]MI-labeled bran with 80% ethanol.

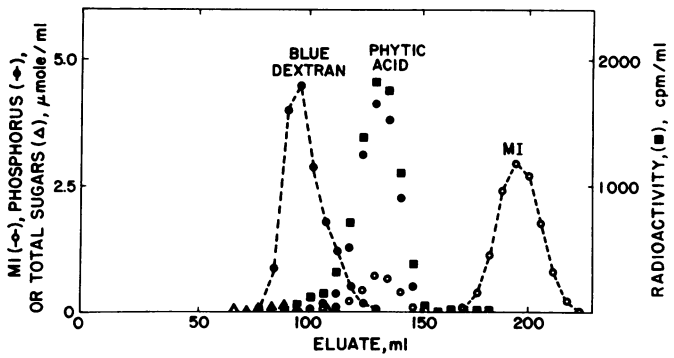

FIG. 4. Sephadex G-25 gel filtration of the EDTA extracted fraction from [2-^{3}H]MI-labeled bran.

was further identified as phytic acid by paper electrophoresis and by chemical analysis for MI and Pi after acid hydrolysis.

The TFAA-soluble fraction from [2-^{3}H]MI-labeled bran was treated with sodium borohydride and partially separated into neutral and acidic components by ion-exchange chromatography. The ratio of ^{3}H between neutral and acidic components was 1:1. The major labeled monosaccharides in the neutral fraction were arabinose and xylose. A detailed analysis of the TFAA fraction was not undertaken since results resembled those obtained in earlier studies with labeled polysaccharides from [2-^{3}H]MI-labeled grain (13).

DISCUSSION

Potential sources of MI for phytic acid biosynthesis and cell wall formation in developing wheat kernels include direct biosynthesis of MI within the kernel from hexose and translocation of MI, free or combined, from biosynthetic sites elsewhere in the

plant. Kurasawa *et al.* (11) isolated MI 1-phosphate synthase from developing rice grains, but further studies on the localization of this enzyme within the kernel or its occurrence in other grain crops are lacking. Failure to obtain conversion of [^{14}C]glucose to phytic acid in dissected aleurone tissue from developing rice grains (15) suggests a site of MI biosynthesis other than the aleurone layer if the kernel is indeed a major source of MI for phytate deposition.

The alternative, translocation of MI from sites of biosynthesis elsewhere in the plant to the developing kernel, is an attractive one. MI is a rather common constituent of sieve-tube exudate (23). Moreover, it is a direct product of galactinol:sucrose 6-galactosyltransferase activity and its presence with galactinol in a wide selection of plant species suggests an active role in raffinose and stachyose biosynthesis (18). Both raffinose and stachyose are found in kernels of wheat, in an amount just slightly less than sucrose (3).

The experimental approach used in the present study focuses attention on translocation of free MI and its conjugate, MI-Gal, which may be formed during transit in the vascular system. With [2-^{3}H]MI as the source of injected label, the major products were cell wall constituents derived from UDP-glucuronate via the MI oxidation pathway, phytate, MI-Gal, and free MI. Cell wall products were deposited in the region surrounding the site of injection, in the outer layers of the kernel, and, to a lesser degree, in all tissues which underwent further cell wall formation or cell expansion above the site of injection. Phytate deposition was limited to the bran fraction of the kernel as was the occurrence of MI and MI-Gal. This localization in the bran fraction, actually the aleurone layer (21), was not encountered when D-[U-^{14}C]glucose or [U-^{14}C]sucrose was used to replace [2-^{3}H]MI as source of injected label. In the ^{14}C-labeling experiments, harvested kernels gave bran, middling, and flour fractions in which ^{14}C was uniformly distributed (S. C. Gumber and F. A. Loewus, unpublished experiments). Further, the carbohydrate constituents of each fraction showed uniform distribution of label. This is consistent with the finding by Jenner and Rathjen (8) regarding uptake and utilization of sucrose by grains of wheat during development. Autoradiographic studies of peduncle-injected, [U-^{14}C]sucrose-labeled developing wheat spikes revealed a uniform distribution of ^{14}C along the crease area of the kernel (17). Here, unreported experiments on the distribution of ^{3}H in [2-^{3}H]MI-labeled kernels at the soft endosperm stage revealed similar values for specific radioactivity in each half-kernel when kernels were divided at right angles to the crease. Results are in agreement with a process of translocation from peduncle to kernel that utilizes the vascular processes, but present evidence does not rule out participation of xylem elements even though, within the kernel, data obtained here as well as that from the studies of Sakri and Shannon (17) are indicative of transport through sieve elements. Autoradiographic studies of pistils of *Lilium longiflorum*, which had been supplied with [2-^{3}H]MI through the severed pedicel, revealed selective labeling of sieve tube walls, whereas xylem wall elements within the same vascular bundles were virtually unlabeled (10).

In the case of MI-labeled kernels, two major sinks, phytate biosynthesis and cell wall formation, drained away MI as it reached the end of its passage through the vascular system. Together, free MI and MI-Gal accounted for only 4% of the ^{3}H recovered in the bran. By contrast, SI, which was not utilized for either phytate biosynthesis or cell wall formation, continued to accumulate as free SI and SI-Gal in outer layers of the kernal until nearly all translocated label was deposited. SI was not toxic to plant growth. Both MI- and SI-injected wheat stalks proceeded through development and ripening indistinguishable in growth and physiology from controls. When 2-*O*,*C*-methylene-MI accompanied by a trace amount of [2-^{3}H]MI was injected, this MI antagonist quickly inhibited kernel growth. Injection of large amounts of MI along with the 2-*O*,*C*-methylene-MI prevented that inhibition. This work on the inhibitory property of 2-*O*,*C*-methylene-MI is still in progress.

Since a portion of the administered label was recovered as its α-galactoside in mature kernels, this glycoside may function as the intermediate form of MI which is utilized by the wheat plant for translocation to the kernel. This study does not rule out possible biosynthesis of the galactoside after it reaches the kernel. In fact, the MI-Gal synthesizing enzyme described by Frydman and Neufeld (6) was obtained from fresh garden peas. Further research is needed to clarify this interesting aspect.

Perhaps the most useful information to emerge from this study is the finding that SI, although not utilized by the plant as a substrate for phytate biosynthesis or the MI oxidation pathway, does mimic MI during translocation. Traces of [2-^{3}H]MI present in the [*R*-^{3}H]SI used in this study interfered with efforts to assess possible epimerization of SI to MI in the course of SI labeling. Within this limit, there was no detectable conversion of SI to MI or its metabolic products. Further assessment will require use of labeled SI that is totally devoid of labeled MI.

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