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Inositol Metabolism in Plants. IV. Biosynthesis of Apiose in Lemna and Petroselinum

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Summary. The biosynthesis of apiose was investigated in cell wall polysaccharide of *Lemna gibba* G3 (duckweed) and in detached leaves of *Petroselinum crispum* (parsley). *Lemna* grown either in short days or in continuous light incorporated ^{14}C from a medium containing *myo*-inositol-2-^{14}C into D-apiosyl and D-xylosyl units of cell wall polysaccharides. Labeled D-apiose was characterized by paper chromatography, by formation of labeled crystalline di-O-isopropylidene D-apiose, and by gas chromatography of trimethylsilyl derivatives of apiose and of its sodium borohydride reduction product, apiitol. Periodate oxidation of labeled apiose revealed 86 to 94 % of the ^{14}C was located in formaldehyde fragments corresponding to C3′ and C4. Comparison of this result with work reported by Grisebach and Doebereiner and by Beck and Kandler supports the conclusion that *myo*-inositol-2-^{14}C was converted to D-apiose labeled specifically at C4.

When L-arabinose-1-^{14}C was supplied to *Lemna*, both L-arabinosyl and D-xylosyl units of cell wall polysaccharides became labeled, but no ^{14}C was found in D-apiose. Analysis of the medium external to the plants revealed the presence of a polysaccharide-like polymer that also contained labeled xylose and arabinose.

Petroselinum leaves utilized *myo*-inositol-2-^{3}H for the synthesis of apiose in apiin.

These results provide direct evidence for a pathway of apiose biosynthesis involving D-glucuronic acid metabolism.

Bell, Isherwood, and Hardwick (7) observed that mild acid hydrolysis of dried fresh leaves or fibers from *Posidonia australis* release free D(+)-apiose in substantial yield. Subsequently, others found D-apiose as a constituent of cell wall polysaccharides in several plant species (4, 5, 13, 28). Prior to the report of Bell et al., only apiin had been recognized as a natural source of this branched chain, 5 carbon sugar (16). The stereochemistry of apiose has been clarified (17, 28).

Biosynthesis of apiose has been studied in apiin (14, 15, 23) and in cell wall polysaccharide from *Lemna* (5, 6, 23). In these investigations, labeled 1 or 2 carbon donors (bicarbonate, formate, serine, methionine, acetate, etc.) or specifically labeled forms of D-glucose were used. After a period of metabolism, apiose was isolated from the plant and assayed for incorporated label. Results obtained from these studies have led to a proposed pathway of biosynthesis that includes features offered earlier for the biosynthesis of streptose (9, 10, 11). The essential features of this pathway, as presented by Beck and Kandler (6) and by Grisebach and Doebereiner (15) are illustrated in figure 1.

This scheme can be tested directly using specifically labeled *myo*-inositol. *myo*-Inositol is an effective precursor of D-glucuronic acid in higher plants and is rapidly and specifically utilized in the biosynthesis of uronic acid and pentose residues common to the cell wall polysaccharides (20, 25, 26). These residues include D-glucuronic acid, 4-O-methyl-

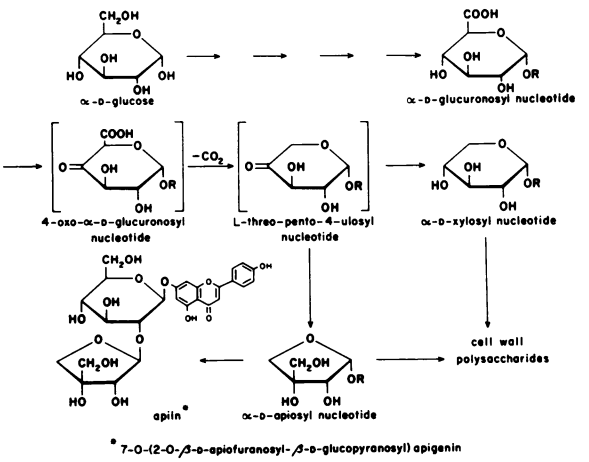

FIG. 1. Apiose biosynthesis as proposed by Beck and Kandler and by Grisebach and Döebereiner.

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D-glucuronic acid, D-galacturonic acid, D-xylose, and L-arabinose. Experiments with specifically labeled precursors have established the nature of this conversion, cleavage of *myo*-inositol between C1 and C6 accompanied by oxidation of C1 to a carboxyl group to form D-glucuronic acid. Subsequent metabolism of D-glucuronic acid accounts for the appearance of specifically labeled uronic acid and pentose residues in cell wall polysaccharides.

The present paper extends these observations regarding *myo*-inositol metabolism to the biosynthesis of apiose from cell wall polysaccharide of *Lemna* and apiin of *Petroselinum*.

Materials and Methods

Lemna *Culture*. Sterile cultures of *Lemna gibba* were grown on liquid E medium with sucrose (1 %, w/v) as recommended by Cleland and Briggs (12). Each experiment was initiated with 4 clones of *Lemna* per 100 ml of medium. Plants grown in short days (9 hrs) were placed at a distance of 30 cm under 2 40-watt cool-white fluorescent tubes plus 2 25-watt incandescent bulbs at 28°. Plants grown in continuous light were similarly placed under 2 40-watt warm-white fluorescent tubes at 25°.

Isolation of Apiose from Lemna. To remove excess culture fluid, plants were filtered on a Buchner funnel, thoroughly washed with portions of distilled water to remove adhering traces of radioactivity, and drained of excess moisture by drawing air through the packed mass of *Lemna* for 15 minutes. Plants were disintegrated in cold 80 % (v/v) ethanol (100 ml/10 g moist tissue) in a Servall Omnimixer, centrifuged, and the residue was re-extracted with portions of 80 % ethanol until washings were free of chlorophyll and contained only traces of ^{14}C. Subsequent extractions with 100 % ethanol, chloroform-methanol (1:2, v/v), and ether left a white powder, referred to hereafter as ethanol insoluble residue.

Apiose was recovered from ethanol insoluble residue by hydrolysis in dilute hydrochloric acid. Others have noted that this treatment released nearly all the apiose but very little of the xylose and glucose present in polysaccharide from *Lemna* and other apiose-rich sources (7, 13, 23). Following hydrolysis and removal of insoluble residue by centrifugation, the supernatant was deionized, evaporated to a small volume, and chromatographed in solvent A (see below). Apiose solutions were assayed for reducing sugar by reaction with alkaline ferricyanide reagent (3). Reducing values identical to those obtained with equivalent amounts of xylose, arabinose, or glucose were obtained from weighed samples of apiose recovered by chromatography from apiin hydrolyzates.

Isolation of Apiose from Petroselinum. Crude apiin was extracted from freshly detached leaves of *Petroselinum crispum* (17). Hydrolysis of apiin in boiling 0.1 N HCl for 1 hour released apiose as free sugar. After filtering the hydrolyzate and evaporating the filtrate to a small volume, the solution was passed through a column of powdered cellulose (63 × 5 cm) with acetone-water (9:1, v/v) as developing solvent. Apiose was recovered in the effluent between 1.5 to 1.8 l. The method outlined here was used to prepare apiose in quantity. When apiose was recovered from labeled *Petroselinum* leaves, apiin was first isolated as its lead salt (23).

Di-O-isopropylidene Apiose. The procedure of Williams and Jones was modified by adding anhydrous copper sulfate (400 mg) and apiose (56 mg) to acetone (3 ml) containing 0.5 % sulfuric acid. The reaction time was extended to 43 hours at 25°. After neutralizing the reaction mixture and filtering, N sodium hydroxide (0.2 ml) was added to the filtrate and acetone was removed in a stream of nitrogen at 25°. Sublimation of the residue at 65° under reduced pressure gave pure di-O-isopropylidene apiose in a yield of about 50 %.

Trimethylsilyl Derivatives of Apiose. Grisebach and Doebereiner (14, 15) have reported on the preparation of these derivatives. They observed 4 peaks in gas chromatograms. Similar results have been obtained in the present study. The course of

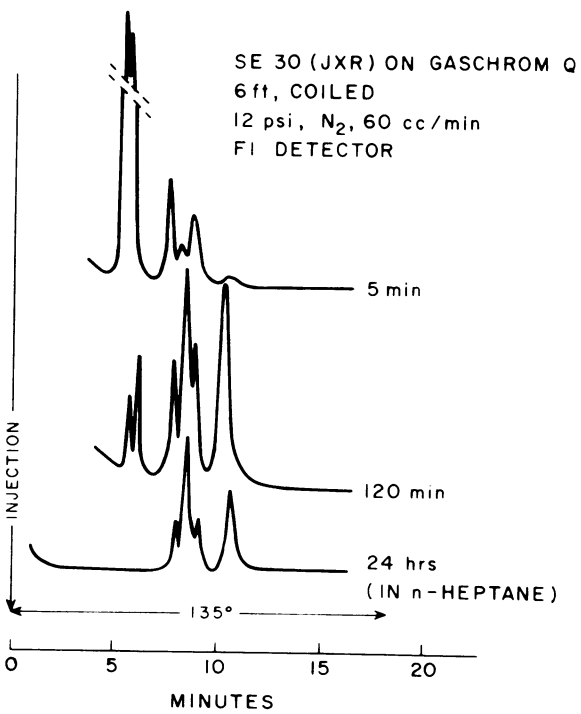

FIG. 2. Gas chromatograms of trimethylsilyl derivatives of D-apiose. Upper 2 plots were made with aliquots of a reaction mixture taken 5 and 120 minutes after mixing. Lower plot was made with a sample extracted from the dried reaction mixture with *n*-heptane 24 hours after mixing.

trimethylsilylation appeared to proceed more slowly than that of other sugars (27). Five minutes after reactants were mixed, the gas chromatograph revealed that most of the product was present in a pair of partially resolved peaks with retention volumes of 6.4 and 6.9 minutes (fig 2). With time, the initial products disappeared and the final pattern of 3 partially resolved peaks (7.9, 8.6 and 9.1 min) and 1 fully separated peak (10.7 min) emerged. There seemed to be a relationship between the increase in the second and fourth peaks in the final chromatogram and the disappearance of the 2 initial peaks. Possibly, the hydroxyl attached to C3 of apiose was substituted more slowly in 1 furanose configuration than in the other.

Trimethylsilyl Derivative of Apiitol. Apiitol was formed quantitatively from apiose by reduction in aqueous solution with excess sodium borohydride. Excess reagent was destroyed with formic acid. Cations were removed by passing the apiitol solution through a column of Dowex 50 H^+ resin and boric acid was removed by repeated evaporation of the Dowex 50 effluent with portions of methanol. Gas chromatography of the trimethylsilyl derivative of apiitol revealed a single peak with a retention volume of 10 minutes shortly after reactants had been mixed. With time, this peak slowly decreased and was replaced by a second peak (retention volume, 14 min). After the reaction mixture had stood 24 hours at 25°, only the peak at 14 minutes remained. Apparently a tetra*kis*-trimethylsilyl derivative is rapidly formed, probably the one unsubstituted at C3, followed by complete trimethylsilylation to the final product, penta*kis*-trimethylsilyl apiitol. Replacement of all 4 peaks in the gas chromatogram of the trimethylsilyl derivatives of apiose by a single peak in the trimethylsilyl derivative of apiitol prepared from the same sample of apiose lends support for the view that the 4 components in apiose are isomers.

Gas Chromatography of Trimethylsilyl Derivatives. Normally, trimethylsilyl derivatives were transferred into *n*-heptane before chromatography. Samples were injected onto a coiled glass column (6 ft) containing 3% JXR on Gas Chrom Q (Applied Science Laboratories, State College, Pa.). The carrier was nitrogen and the effluent was monitored with a flame ionization detector. For derivatives with retention volumes similar to or less than that of rhamnitol, the column temperature was 135°. Samples with higher retention volumes were programmed at 5° per minute from 135° to 240°. Radioactive samples were recovered for counting by diverting the larger portion of a 7:1 stream splitter through a trap held at −70° or, in certain experiments, by flow counting (18).

Assay of Radioactivity. Chromatograms were scanned on a Packard Model 7200 scanner. Single samples were dissolved in water (0.5 ml) and added to a liquid scintillation mixture (15 ml) of naphthalene (100 g), PPO (7 g), and dimethyl POPOP (0.3 g) in *p*-dioxane to make 1 liter. Samples were counted in a Packard Model 3000 spectrometer with an efficiency of 78% for ^{14}C and 22% for ^{3}H.

Chromatography. The following solvent systems were used. A) Ethyl acetate-water-acetic acid-formic acid (18:4:3:1, v/v). B) Water-saturated phenol. C) Butanol-pyridine-water-benzene (5:5:3:1, v/v). D) Ethyl acetate-pyridine-water-acetic acid (5:5:3:1, v/v). E) Ethyl acetate-pyridine-water (8:2:1, v/v). F) Methyl ethyl ketone-butanol-acetic acid-water (3:2:2:2, v/v).

$R_{glucose}$ values on paper for xylose and apiose respectively were A) 1.34, 1.73; B) 1.33, 1.44; C) 1.35, 1.67; D) 1.16, 1.27; and E) 1.84, 2.75 and for arabinose C) 1.1 and E) 1.53. R_F values on thin layer, cellulose plates for xylose and apiose were B) 0.34, 0.52; D) 0.52, 0.61; and F) 0.39, 0.46. Sugars were detected with silver nitrate in aqueous acetone followed by alcoholic KOH or by a mixture of aniline and trichloroacetic acid followed by brief heating at 100°. With the latter, apiose gave a bright yellow color, straight chain pentoses, pink, and methyl pentoses, brown.

Results and Discussion

Biosynthesis of Apiose in Lemna. Table I contains a summary of results from 3 experiments. Evidence for the incorporation of ^{14}C from *myo*-inositol-2-^{14}C into apiose and xylose units of cell wall polysaccharide was obtained in experiments 1 and 2. Experiment 3, in which *Lemna* were labeled with L-arabinose-1-^{14}C, was included in order to test the possibility that xylose units of cell wall polysaccharides could be labeled exclusively. Previous studies (22, 24) had shown that L-arabinose is readily utilized by higher plants as a source of D-xylosyl and L-arabinosyl units of cell wall polysaccharides. Neither Mendicino and Picken nor Beck and Kandler mentioned the presence of L-arabinose in their cell wall hydrolyzates from *Lemna*.

Hydrolysis of ethanol insoluble residue (4 mg) from experiment 1 in N HCl (2 ml) for 1 hour at 100° gave a solution containing glucose, xylose, and apiose. Insoluble residues were filtered off and the filtrate was evaporated to dryness. Only a portion of this dried material could be redissolved in water. A considerable part of the ^{14}C remained undissolved. When the soluble portion was chromatographed on paper in solvent A (fig 3a), the greatest proportion of ^{14}C remained on the origin (4400 dpm) with the remaining radioactivity appearing in 2 spots corresponding to xylose (1250 dpm) and apiose (1700 dpm). Glucose was not labeled.

Another portion (12 mg) of ethanol insoluble residue from experiment 1 when hydrolyzed in

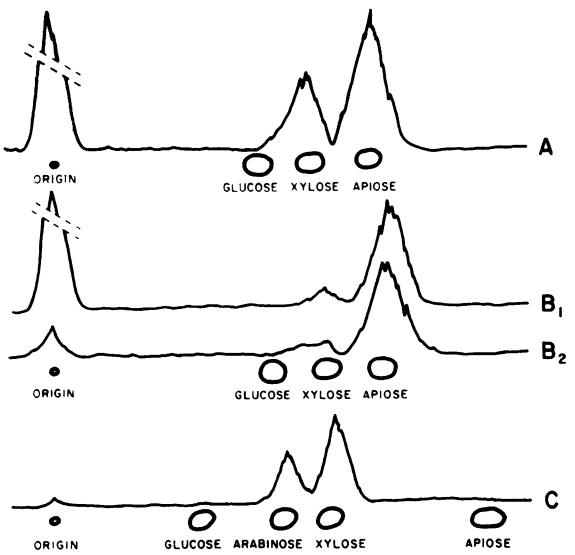

FIG. 3. Radiochromatograms of cell wall hydrolyzates from *Lemna*. 3a, $3b_1$, and $3b_2$) Hydrolyzates of ethanol insoluble residues labeled with *myo*-inositol-2-^{14}C (developed in solvents A, B, and B respectively). 3c) A hydrolyzate of ethanol insoluble residue labeled with L-arabinose-1-^{14}C (developed in solvent E). See text for further experimental detail.

0.1 N HCl (1 ml) for 0.5 hour at 100° released about one-third (18,600 dpm) of the ^{14}C in the residue. The hydrolyzate, freed of insoluble matter, was divided into 2 parts, 1 of which was chromatographed on paper in solvent B without further treatment (fig $3b_1$). The other part was deionized by passage over columns of Dowex 50 H^+ and Dowex 1 formate resin before chromatography in solvent B (fig $3b_2$). Comparison of the 2 radiochromatogram scans (fig $3b_1$ and $3b_2$) showed that deionization removed practically all of the ^{14}C present at the origin. The deionized hydrolyzate (5110 dpm) contained labeled apiose as its major constituent, traces of labeled xylose, and a faint chemical trace of unlabeled glucose. The nature of the labeled substances retained by ion exchange resins is still under study.

Of the total ^{14}C supplied as *myo*-inositol-2-^{14}C in experiments 1 and 2, 1.7 and 1.6 % respectively were recovered in apiose. In terms of ethanol insoluble residue, the corresponding values were 8 and 7 %. The specific activities of apiose recovered in these experiments were 0.3 and 0.57 μc/mmole, respectively. Characterization and degradation of apiose are discussed in another section.

Experiment 3, in which L-arabinose-1-^{14}C was supplied to *Lemna*, resulted in massive incorporation of label into cell wall polysaccharides. When a portion (7.6 mg) of ethanol insoluble residue was hydrolyzed in N hydrochloric acid (5 ml) for 0.5 hour at 121° in a sealed tube, 82 % (45,000 dpm) of the ^{14}C was solubilized. Chromatography in solvent C or E (fig 3c) revealed that all of this soluble ^{14}C was present as arabinose (36 %) and xylose (64 %). Neither apiose nor glucose were labeled although both were present in expected amounts.

Although considerable ^{14}C remained in the final medium (table I) used to grow labeled *Lemna* only the nature of labeled substances present in that of experiment 3 has been sufficiently characterized to report at this time. After reducing the volume of the medium to a few ml, a portion (0.14 μc in 3 ml) was fractionated on a column (32 × 2.5 cm) of Sephadex G-50. Both ^{14}C and orcinol reactive substance appeared as a sharp peak (fig 4) in the excluded volume which for G-50 corresponds to molecules with a weight over 10,000. The polysaccharide nature of this excluded material was demonstrated by hydrolyzing a portion in N hydrochloric acid for 0.5 hour at 121° (sealed tube). When the hydrolyzed material was chromatographed in solvent E and scanned for ^{14}C, 3 regions, one at the origin, another corresponding to arabinose, and the last to xylose were detected. The relative ratio of ^{14}C under the peaks was 1:5.8:1.6. Interfering color reactions prevented pentose assays beyond the first 100 ml of eluate. Chromatography in solvent E of ^{14}C containing material from the second radio-

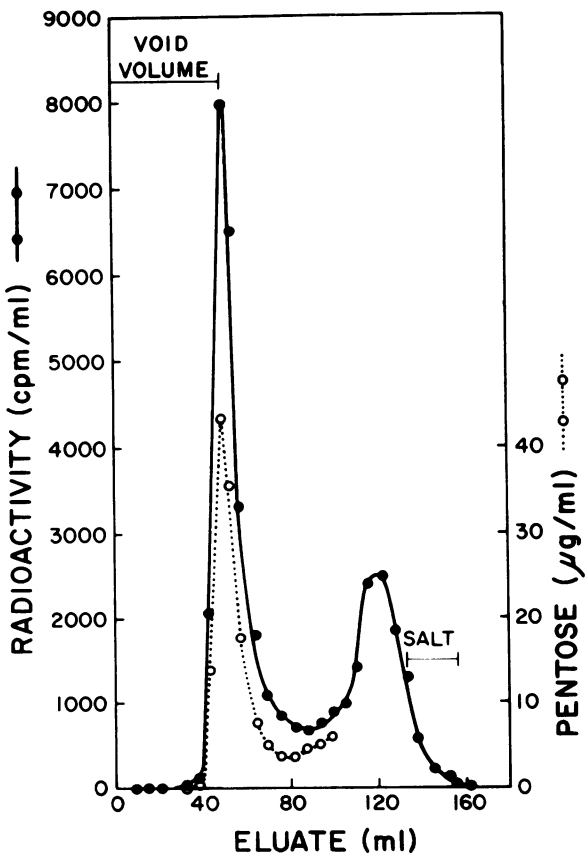

FIG. 4. Separation on Sephadex G-50 of labeled constituents present in the final growth medium of *Lemna* labeled with L-arabinose-1-^{14}C.

Table I. *Distribution of* ^{14}C *in* Lemna *Grown in Medium Containing* myo-*Inositol-2-*^{14}C *or* L-*Arabinose-1-*^{14}C

Labeled substrate	Expt 1* *myo*-Inositol-2-^{14}C	Expt 2 *myo*-Inositol-2-^{14}C	Expt 3* L-Arabinose-1-^{14}C
μmoles/100 ml of medium	0.47	0.37	4.0
μc/100 ml of medium	5.5	4.1	2.6
Growth, weeks	18	4	8
Ethanol insoluble residue recovered, g/100 ml of medium	0.5	0.45	0.61
Distribution of ^{14}C, μc			
Final medium	1.86	1.48	0.25
80 % Ethanol extractions	2.04	1.07	0.10
Subsequent extractions**	0.07	0.03	negligible
Ethanol insoluble residue	1.16	0.92	2.09
^{14}C not recovered***	0.4	0.6	0.2
Apiose	0.09	0.065	none

* Short day (9 hrs.)
** See text.
*** By difference.

active peak gave no labeled components beyond the origin. However, when a portion of this material was hydrolyzed under conditions comparable to those used for the material in the excluded volume, a portion of the ^{14}C was released as arabinose and xylose.

The origin of this polysaccharide-like material found in the final medium of L-arabinose-1-^{14}C labeled *Lemna* is undetermined. Possible sources include extracellular processes, secretion by fronds or root-like tissues, or cellular decomposition. Certainly, elaboration of polysaccharides with the composition encountered in this experiment could have considerable biological significance in the normal aquatic environment of this plant.

A portion of ^{14}C administered as *myo*-inositol-2-^{14}C or L-arabinose-1-^{14}C was recovered neither in *Lemna* tissue nor in the final medium. Presumably, this radioactivity was lost as respired $^{14}CO_2$ although no attempt was made to trap or to measure this gas.

Biosynthesis of Apiose in Petroselinum. Six young leaves (8 g), freshly detached, were transferred to a single vial containing 0.05 M *myo*-inositol-2-^{3}H (0.5 ml, 150 μc). When the labeled solution was depleted (6 hrs), successive portions of distilled water were added (72 hrs). Apiin (2.5 μc), recovered from an ethanol-water extract of the leaves as its lead salt, was hydrolyzed and chromatographed in solvent C. Neither the aglycone, apigenin, nor the glucose moiety contained measurable tritium.

Labeled apiose was converted to its trimethylsilyl derivative and further purified by gas chromatography. Retention volumes corresponding to the 4 peaks of apiose were trapped at −70°, hydrolyzed with ethanol-water (1:1, v/v), and concentrated to a volume of a few μl. Aliquots (1 μl, 216 cpm) were applied as spots to a thin layer cellulose plate along with controls, including one prepared from a hydrolyzate of *Lemna* cell wall, and developed in solvent D. A portion of the plate was sprayed with benzidine-trichloroacetic acid (13) to locate the apiose region. Cellulose powder scraped from 1 cm^2 of the apiose region of a single aliquot contained 193 cpm. When a similar region was combined with that from a *Lemna* control (as carrier) and converted to its trimethylsilyl derivative, gas chromatography gave a typical apiose pattern and the radioactivity of the retention volume containing this derivative accounted for 184 cpm.

Characterization and Degradation of D-*Apiose.* Ethanol insoluble residue (69 mg) from experiment 2 was hydrolyzed in 0.1 N hydrochloric acid. The deionized hydrolyzate, freed of insoluble residues, contained 25 to 30 % of the ^{14}C released by acid. Apiose (0.56 μc/mmole) was recovered from the hydrolyzate by chromatography in solvent A. A portion (0.32 mg) was diluted with unlabeled D-apiose (56.3 mg) from apiin and converted to its di-O-isopropylidene derivative; yield after sublimation, 38 mg; melting point, 79 to 80°; $[\alpha]_D$ +59.3° (C 1.02 in ethanol); specific activity, 7400 dpm/mmole (calculated specific activity, 7100 dpm/mmole). Williams and Jones (28) obtained a melting point of 80 to 82° and $[\alpha]_D$ +58° (C 1.17 in ethanol) for the same derivative of D-apiose from *Zostera marina* L. Gas chromatography of di-O-isopropylidene D-apiose on a column (see Methods) at 90° gave a single asymmetric peak (11.5 min).

A portion (770 dpm) of the di-O-isopropylidene D-apiose prepared from *Lemna* labeled with *myo*-inositol-2-^{14}C was hydrolyzed to free D-apiose and then oxidized with 4 equivalents of sodium periodate. The procedure of Bloom (8) was used to trap formaldehyde as its dimedone derivative from the reaction mixture. After recrystallization from ethanol-water, the derivative melted at 190° (corr.). A portion (14.5 mg) was counted and found to contain 122 dpm corresponding to 2460 dpm/mmole of formaldehyde. Since the oxidation resulted in 2 molecules of formaldehyde per molecule of apiose

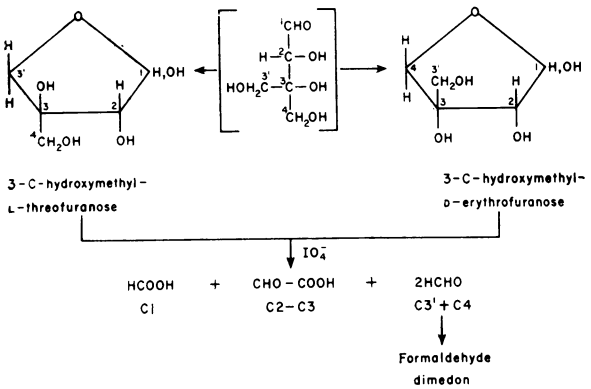

FIG. 5. Periodate oxidation of D-apiose.

(fig 5), recovery of ^{14}C as the dimedon derivative accounted for 86 % of the label in apiose, located either in C3′, C4, or both.

In another experiment, apiose (0.16 mg) from *myo*-inositol-2-^{14}C labeled *Lemna* (expt 2) was diluted with apiin-derived apiose (15 mg) and then oxidized with 4 equivalents of sodium periodate (8). The dimedon derivative of formaldehyde (17.4 mg) contained 282 dpm corresponding to 4850 dpm/mmole of formaldehyde or 94 % of the specific activity anticipated if all ^{14}C in apiose was located in C3′, C4 or both.

Due to the very substantial contributions of Grisebach and Doebereiner (15) and of Beck and Kandler (6), it is possible to chose between the 2 carbons of apiose contributing to formaldehyde in the periodate oxidation. They have concluded from their degradation studies of apiose isolated from plants labeled with D-glucose-3,4-^{14}C as well as D-glucose-6-^{14}C-4-^{3}H and D-glucose-U-^{14}C-3-^{3}H (14) that C3 of D-glucose is the most probable source of C3′ of D-apiose. A similar conclusion had been advanced by Bruton and Horner (9) and by Candy and Baddiley (10) for the biosynthesis of streptose in streptomycin. Although these results did not provide an unequivocal choice between C3 and C4 of D-glucose as precursor of C3′ of D-apiose, the present work with *myo*-inositol-2-^{14}C as source of label, combined with observations just reviewed allows such a choice. The stereochemical relationship of the carbon chain of D-glucose to that of *myo*-inositol was clearly established by studies on the biosynthesis of *myo*-inositol in plants (1, 20). Oxidation of *myo*-inositol-2-^{14}C or -2-^{3}H to uronic acid and pentose products labeled exclusively in C5 had also been demonstrated in plants (19–21). In experiment 2, above, a portion (0.33 mg) of the xylose recovered from ethanol insoluble residue by acid hydrolysis was mixed with unlabeled D-xylose (50 mg). The diluted xylose had a specific activity of 3300 dpm/mmole. A portion (30.3 mg) of the diluted xylose was oxidized with 4 equivalents of sodium periodate. Formaldehyde, recovered as its dimedon derivative (31.4 mg) contained 273 dpm corresponding to 3200 dpm/mmole. Since C5 of xylose was the only carbon recovered as formaldehyde, this activity corresponded to 97 % of the ^{14}C present in the pentose. Thus, the same experimental run used to obtain labeled D-apiose also contained D-xylose labeled almost exclusively in C5. This result establishes the stereospecific nature of *myo*-inositol-2-^{14}C metabolism to pentose in *Lemna*. Since neither C3 nor C4 of the 6 carbon precursor, presumably D-glucuronic acid, was labeled and since others who have been studying apiose biosynthesis clearly showed incorporation of label from C3 and C4 of D-glucose into C3 and C3′ of D-apiose, the specific carbon of apiose labeled by *myo*-inositol-2-^{14}C was C4.

Arabinose in Ethanol Insoluble Residue of Lemna *Labeled with* L-*Arabinose-1-*^{14}C. Carbon-14 equivalent to about one-fourth of the radioactivity supplied as L-arabinose-1-^{14}C was recovered as arabinose from ethanol insoluble residue of experiment 3 (table I). To further establish the chemical nature of labeled as well as unlabeled monosaccharide residues present, a portion of the deionized hydrolyzate was reduced with sodium borohydride and then converted to a mixture of the corresponding trimethylsilyl derivatives. Comparisons were made between the retention volume of each component in the gas chromatograph and the ^{14}C content of each component as monitored on the column effluent with a flow counter. Methanol-^{14}C was used to synchronize retention volumes with the radioisotope scan. The result of such a run is shown in figure 6. Components identified by arrow in this figure were compared with authentic samples. Although the trimethylsilyl ethers of apiitol and arabinitol were only partially resolved, only the arabinitol portion contained ^{14}C. All of the

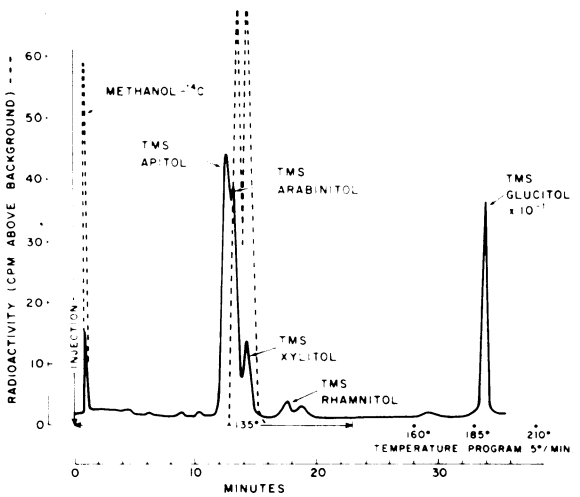

FIG. 6. Gas radiochromatogram of trimethylsilyl derivatives of components from a sodium borohydride-reduced hydrolyzate of ethanol insoluble residue of *Lemna* grown in L-arabinose-1-^{14}C containing medium.

^{14}C in the injected sample appeared in 2 narrow partially resolved peaks corresponding to arabinitol and xylitol. This result confirms paper chromatographic evidence presented earlier. At this time it is not possible to state whether arabinose found in hydrolyzates of ethanol insoluble residue was present only in *Lemna* grown in the presence of L-arabinose or whether it is a normal constituent of the cell wall. Neither Mendicino and Picken nor Beck and Kandler reported the presence of arabinose in *Lemna* cell walls although the latter authors did find rhamnose, an observation confirmed here (fig 5).

Although L-arabinose was readily incorporated into *Lemna* cell wall polysaccharides accompanied by epimerization to D-xylosyl units, no conversion to D-apiosyl units from L-arabinose was observed. Such a conversion would require an intermediate common to apiosyl and xylosyl nucleotides, a suggestion (6) already referred to in the introduction. Although such an intermediate may exist, it might be enzyme-bound, as suggested by Ankel and Feingold (2), and proceed only in the direction of D-xylosyl nucleotide formation.

Additional Comments. To identify apiose, paper chromatography in solvent systems similar to those used in this study is not a sufficient criterion. Bell et al. and others have drawn attention to this but it is a point that should be re-emphasized, particularly since rhamnose, which has a mobility quite similar to apiose, appears in *Lemna* hydrolyzates. Moreover, free D-apiose has 4 possible isomeric configurations as D-apiofuranose. The structures, ignoring anomeric isomers, are given in figure 5 together with the nomenclature specific for each structure. Each structure may have physical or chemical characteristics independent of the other. The isotopic evidence gained in this study as well as previous work by others suggests that 3-C-hydroxymethyl D-erythrofuranose is the form of D-apiose present in glycosides and polysaccharides in plants. We have also noted, in the course of this work that apiose chromatographed on thin layer cellulose plates with solvent C in which water was replaced by water saturated with boric acid (7) is partially resolved into 2 apiose-reactive spots.

Addendum

Grisebach and Sandermann (29) recently reported that label from D-glucuronic acid-U-^{14}C incorporated specifically into the D-apiose moiety of apiin in parsley. The specific activity of C3′ of apiose was one-fifth of that of the whole pentose indicating uniform labeling. When D-glucuronic acid-6-^{14}C was used as a source of label, the ^{14}C content of apiin-derived apiose was less by a factor of 200. These observations further corroborate the scheme proposed earlier (6, 15). It is of interest to note that while Grisebach and Sandermann obtained 0.12 % incorporation of ^{14}C from D-glucuronic acid-U-^{14}C into apiose, in the present paper a comparable experiment in which *myo*-inositol-2-^{3}H was used resulted in 1.7 % incorporation.

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