The Biosynthesis of D-Apiose in *Lemna minor*

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SUMMARY

*Lemna minor* incorporates radioactivity from D-glucose-1-14C, D-glucose-2-14C, D-glucose-3,4-14C, D-glucose-6-14C, sodium acetate-2-14C, DL-serine-3-14C, and L-methionine-14CH3 into the D-apiose moiety of cell wall polysaccharide. The mechanism of biosynthesis of this hydroxymethyltetrose was studied by determining the distribution of 14C in D-apiose after feeding various labeled precursors.

Labeled D-apiose was isolated from this tissue, and an aliquot was reduced to D-apititol. These two compounds were then oxidized with periodate to formic acid, formaldehyde, and glycolic acid, and the radioactivity in each of the carbon atoms of D-apiose was determined.

The results show that D-glucose can serve as a precursor for all of the carbon atoms of D-apiose in *L. minor*, whereas acetate and DL-serine are probably converted to D-glucose before being incorporated into D-apiose. The evidence obtained in this study indicates that D-apiose is derived from D-glucose by the loss of carbon atom 6, followed by a branching reaction which occurs without randomization, and converts either C-3 or C-4 of D-glucose to one of the hydroxymethyl groups of D-apiose.

Apiose (3-C-hydroxymethyl-D-tetrose) was first isolated from parsley plants (*Apium petroselinum*) as a component of the glycoside apiin (1). It has since been shown to occur in a wide variety of plants, including *Lemna minor*, as a component of cell wall polysaccharide (2-5).

Previous studies with parsley and *L. minor* established that carbon atoms 1 through 4 of D-glucose were incorporated into D-apiose, whereas carbon atom 6 of D-glucose was not utilized (6). The C-hydroxymethyl group may have been derived from either C-3 or C-4 of D-glucose (7). Radioactive compounds such as acetate-1-14C (7), acetate-2-14C (6), formate-14C (7), and L-methionine-14CH3 (6) were also effective precursors of D-apiose, indicating that a 1-carbon donor might also be involved in the synthesis.

Several mechanisms have been proposed for the formation of branched chain sugars. Hough and Jones have suggested that an aldol condensation between dihydroxyacetone and glycolaldehyde could form D-apiose (8). Shemyakin, Khokhlov, and Kolo-
ssov proposed a general mechanism for branched chain sugar synthesis, involving a cyclic intermediate (9). Candy, Blumson, and Baddiley have proposed a mechanism involving a transannular rearrangement of a hexose derivative during the formation of streptose (10).

The results reported in the present communication indicate that D-glucose is converted to D-apiose by the loss of 1 carbon atom and the subsequent conversion of a straight chain sugar to a branched chain isomer. A method for the degradation of D-apiose and the determination of 14C in each carbon atom of this sugar is described.

EXPERIMENTAL PROCEDURE

Materials and General Methods—Commercial preparations were used unless otherwise specified. The radioactive substrates were purchased from New England Nuclear. D-Apiose and D-apiose-14C were prepared from *L. minor* by the method previously described (6). *L. minor* was obtained from Verco Tropical Fisheries, Columbus, Ohio.

Radioactivity measurements were made in a Packard Tri-Carb liquid scintillation spectrometer by using a solvent system consisting of 2 volumes of toluene, containing 0.4% 2,5-di-phenyloxazole (PPO) and 0.01% 1,4-bis[2-(5-phenyloxazolyl)]-benzene (POPOP), to 1 volume of Triton X-100 (Rohm and Haas) (11). The system had an efficiency of 40%.

The following solvents were used for the identification and quantitative analysis of sugars and sugar alcohols by paper chromatography: 1-butanol-pyridine-water, 6:4:3 (12); ethyl acetate-acetic acid-water, 3:3:1 (13); acetic acid-1-butanol-water, 1:4:1 (14); 1-butanol-ethanol-water, 52:32:16 (14); 1-butanol-acetic acid-water, 7:1:2 (16). The use of alkaline solvents such as 1-butanol-ethanol (4:1) saturated with borate buffer, pH 8.9 (17), or paper electrophoresis of sugars with borate buffer (18) resulted in the destruction of D-apiose. The free sugar is very labile in alkaline solvents (19). The silver nitrate and alcoholic NaOH reagent (20) and an anisidine reagent (21) were used to detect compounds on the paper chromatograms, and radioactive D-apiose was detected by autoradiography with Kodak “no screen” medical x-ray film.

The concentration of D-apiose was determined by the method of Somogyi (22) and Nelson (23), or by a modification of the method of Dische and Dische (24) for the assay of tetroses with D-fructose and sulfuric acid.

Incubation of Labeled Compounds with *L. minor*—In each experiment, 2.5 g of fresh *Lemna* were blotted dry and placed in a layer over the base of a Petri dish 9 cm in diameter. The appropriate radioactive substrate, dissolved in 5 ml of water, was

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in 2 ml of water were added to a solution containing 45 mg of D-apiose was isolated and purified by procedures described previously in a single, well-defined region with an Rp identical with isolated by paper chromatography with three solvent systems deionized solution was concentrated to a syrup. n-Apiose was through Dowex 50-H+ and Dowex 1-acetate columns, and the reaction mixture was adjusted to pH 6 with 1 N acetic acid. The apiose in 10 ml of water. After 2 hours at room temperature, the periodate was stopped by drying the tissue with absorbent paper and exhaustively extracting it with boiling methanol. The residue was dried under reduced pressure over silica gel.

Isolation of d-Apiose and Preparation of d-Apiitol—The d-apiose was isolated and purified by procedures described previously (6). The dried residue obtained from the Lemma was hydrolyzed with 20 ml of 0.1 N H2SO4 at 100° for 20 min. Insoluble material was removed from the hydrolysate and washed with water. The filtrate and washes were combined and passed through Dowex 50-H+ and Dowex 1-acetate columns, and the deionized solution was concentrated to a syrup. d-Apiose was isolated by paper chromatography with three solvent systems (12-14). In each case the sugar was recovered virtually quantitatively in a single, well-defined region with an Rp identical with that of d-apiose. The yield of d-apiose by this procedure was about 10 mg from 2.5 g of fresh L. minor. Sufficient unlabeled d-apiose, approximately 80 mg, was added as carrier for the degradation reactions and the determination of the pattern of labeling with 14C. Further support for the radioactivity of the isolated d-apiose was obtained by preparing derivatives of some of the diluted samples. The chromatographically pure d-apiose, the phenylazosone (m.p. 154-156°) (19), and the sugar obtained from a crystalline di-o-isopropylidene (m.p. 80-83°) derivative (19) had essentially identical specific activities.

For the preparation of d-apitol, 20 mg of sodium borohydride in 2 ml of water were added to a solution containing 45 mg of d-apiose in 10 ml of water. After 2 hours at room temperature, the reaction mixture was adjusted to pH 6 with 1 N acetic acid. The solution was passed through a column of Dowex 50-H+, and boric acid was then removed by codistillation with methanol. The final residue was dissolved in 10 ml of water and passed through a column of Dowex 1-acetate. The d-apitol was isolated by paper chromatography with two solvent systems (15, 16). The d-apitol was nonreducing in the Somogyi-Nelson test and yielded essentially 2 moles of formic acid, 1 mole of glycolic acid, and 1 mole of formic acid after periodate oxidation. More than 80% of the labeled d-apiose was recovered as d-apitol by this procedure, as determined by the amount of 13C present in the chromatographically pure sample.

Periodate Oxidation of d-Apiose and d-Apiitol—All solutions were cooled to 2° before mixing, and the oxidation was performed in the dark at 2°. The periodate uptake was measured spectrophotometrically (25, 26). One millilitre of 1 M sodium metaperiodate buffer, pH 3.6, and 2 ml of 0.05 M sodium metaperiodate solution were added to 1.5 mg of d-apiose or d-apitol dissolved in 2 ml of water. Aliquots of 0.1 ml were diluted to 25 ml, and the absorption was measured at 220 ma. The periodate uptake was determined by comparing the optical density of these samples with equimolar solutions of sodium metaperiodate and sodium iodate.

A simultaneous determination of formaldehyde was carried out by a modification of the chromotropic acid method (27). The reagent was prepared by dissolving 1 g of the sodium salt of chromotropic acid (4,5-dihydroxy-2,7-naphthalenedisulfonic acid) and 0.1 g of stannous chloride in 100 ml of water, and diluting the solution to 500 ml with concentrated H2SO4. One millilitre of 5% sodium sulfate solution and 10 ml of chromotropic acid reagent were added to 0.1-ml samples of the reaction mixture. After the samples had been heated for 30 min in a boiling water bath, the absorption was determined at 570 mμ. A standard solution of formaldehyde was prepared by treating 1.8 mg of d-glucose, dissolved in 5 ml of 1.0 M NaHCO3 solution, with 4 ml of 0.03 M sodium metaperiodate. The amount of acid formed was determined after a 7.5-μg sample of d-apiose or d-apitol in 10 ml of water was oxidized with 75 mg of sodium metaperiodate for 1 hour. One millilitre of ethylene glycol was added, and after 15 min the solution was titrated potentiometrically with 0.01 N NaOH to an end point of pH 0.25. The total amount of acid plus alkali-labile esters formed was also determined on a reaction mixture identical with that described above. Instead of direct titration of the acid, 25 ml of 0.01 N NaOH were added, and after 1 hour the excess alkali was determined by titration with 0.01 N potassium hydrogen phthalate.

Glycolic acid was determined by the method of Calkins (28) with 2,7-dihydroxy-2,7-naphthalenedisulfonic acid reagent (29). Sodium metaperiodate (27.4 mg in 0.5 ml of water) was added to 2.8 mg of d-apiose or d-apitol in 1 ml of water. Aliquots were taken at various times and treated with barium acetate to precipitate isotope and periodate ions, and were then passed through a Dowex 50-H+ column. The glycolic acid solution used to prepare a standard curve was also treated with sodium metaperiodate and barium acetate under the same conditions as described for d-apiose.

Carbon dioxide formation was measured manometrically. The Warburg flask contained 1.5 mg of d-apiose dissolved in 4 ml of 0.25 M sodium acetate buffer, pH 3.6. After temperature equilibration, 1 ml of 0.06 M sodium metaperiodate solution was added from the side arm.

Degradation of d-Apiose-14C and d-Apioit-14C—A 20-ml solution containing 45 mg of labeled d-apiose or d-apitol and 475 mg of sodium metaperiodate was kept in the dark at 2° for 2 hours. The solution was then passed through a column of Dowex 1-acetate (5 × 1.5 cm), and the resin was washed with water. For the isolation of formic acid, 50 ml of 0.4% dimedone were added to the effluent from the ion exchange column, and the solution was adjusted to pH 4.5 and allowed to stand overnight. Formaldemethone formed was recrystallized from water, m.p. 190-192°.

The formic and glycolic acids which were absorbed on the Dowex 1-acetate column were eluted with 200 ml of 1.0 N acetic acid (29). The locations of these acids in the column effluents were determined by colorimetric assays (28, 30).

The fractions containing formic acid were pooled and concentrated. The formic acid was then isolated as the p-bromophenacyl derivative, and this compound was purified by thin layer chromatography (30).

Fractions from the Dowex 1-acetate column containing glycolic acid were further purified by paper chromatography with a solvent system containing 1-butanol, 95% ethyl alcohol, water, and diethylamine (80:10:20:1) (31). The crystalline phenylhydrazine salt of the glycolic acid purified by this procedure had the same specific activity as the chromatographically pure sample. The glycolic acid was then degraded to carbon dioxide and formic acid with ceric sulfate (32). The carbon dioxide formed during the oxidation of glycolic acid was swept by a continuous stream of nitrogen into an absorption vessel containing sodium hydroxide and was precipitated as barium carbonate, and its specific
activity was measured (32). The formic acid obtained from the oxidation of glycolic acid was removed from the reaction mixture by steam distillation, and the neutralized solution was concentrated. The formic acid was isolated as carbon dioxide by oxidation with mercuric sulfate (32). The specific activity of the formic acid was also measured by isolating the 4-bromophenylacetyl derivative (30) from aliquots of the same samples. The two procedures yielded essentially identical specific activities for the isolated formic acid.

Some compounds isolated during the degradation procedure were diluted with the corresponding nonradioactive material to increase the amount available for subsequent degradation.

RESULTS AND DISCUSSION

Periodate Oxidation of D-Apiose and D-Apiitol—When D-apiose was oxidized with sodium metaperiodate, there was a rapid uptake of 2 moles of periodate per mole of D-apiose, followed by a somewhat slower uptake of 1 mole of periodate. The oxidation of D-apiitol resulted in a very rapid uptake of 3 moles of periodate per mole of D-apiitol. In both cases the oxidation was accompanied by the release of 1 mole of glycolic acid per mole, as shown in Table I. The yield of products obtained after 1 hour is summarized in this table. The oxidation of 1 mole of D-apiose resulted in the formation of 1 mole of formaldehyde, 1 mole of glycolic acid, and 2 moles of formic acid. The same products were obtained in the oxidation of D-apiitol, but 2 moles of formaldehyde and 1 mole of formic acid per mole of apiitol were formed in this case. No carbon dioxide was formed from either compound.

Consideration of the mechanism by which D-apiose is oxidized with periodate is complicated by the fact that there are four possible cyclic forms in addition to the free aldehyde and aldehydrol forms of the sugar. All of these may exist in aqueous solution. A greater release of acid was observed when it was determined by back-titration, rather than by direct titration (Table I). This indicates that a stable ester may have been formed during the oxidation of the cyclic hemiacetal form of D-apiose at a neutral or slightly acid pH (33). Cleavage between C-2 and C-3 of D-apiose will predictably take place more rapidly with the cis-diol (D-apio-D-furanose) form than with the trans-diol (D-apio-L-furanose) form (34). In a study of the periodate oxidation of apiin, Hulyalkar, Jones, and Perry (35) obtained evidence indicating that the initial attack by periodate on the D-apiose moiety resulted in a 35% cleavage of the C-3—C-4' bond and a 65% cleavage of the C-2—C-3 bond. The carbon atom of the hydroxymethyl group involved in the hemiacetal linkage is designated C-4, while that which lies outside the plane of the ring is designated C-4', as shown in Fig. 3. The rate of uptake of periodate and the rate of release of formaldehyde indicate that a similar dual cleavage may be occurring in the oxidation of free D-apiose, as illustrated in the reaction sequence shown in Fig. 2.

The periodate oxidation of D-apiose and D-apiitol, derived from the same sample, provided a convenient means of determining the distribution of 14C in each of the carbon atoms of this sugar. It was possible to correlate the carbon atoms of each of the products formed with the carbon atoms of D-apiose from which they were derived, as shown in Fig. 3.

![Fig. 1. The rate of periodate uptake and formation of formaldehyde when D-apiose or D-apiitol was oxidized with metaperiodate. Curve A, periodate uptake with D-apiitol; Curve B, periodate uptake with D-apiose; Curve C, formaldehyde formed from D-apiitol; Curve D, formaldehyde formed from D-apiose.](http://www.jbc.org/)

![Fig. 2. Proposed reaction sequence for the oxidation of D-apiose by sodium metaperiodate.](http://www.jbc.org/)
Metaperiodate.

rate of synthesis of D-apiose for the formation of cell wall poly-
from D-glucose. Although more D-glucose-3,4\textsuperscript{14}C was fed
only about one-third as effective (Table II). These results are
parsley plants (6), and suggest that D-apiose is derived directly
Metabolism of D-glucose-1\textsuperscript{14}C, D-glucose-2\textsuperscript{14}C, and D-glucose-
3,4\textsuperscript{14}C by
Lemna was incubated in the presence of specifically labeled
L-Methionine-l\textsuperscript{14}CH\textsubscript{3}
N-Serine-3\textsuperscript{14}C 2.0
Glucose-6\textsuperscript{14}C, 10.0
Glucose-1\textsuperscript{14}C 3.2
Glucose-2\textsuperscript{14}C 3.3
Glucose-3,4\textsuperscript{14}C 16.4
Glucose-6\textsuperscript{14}C 1.5
Serine-3\textsuperscript{14}C 2.0
L-Methionine-l\textsuperscript{14}CH\textsubscript{3} 0.7
Sodium acetate-2\textsuperscript{14}C 22.2

Incorporation of \(^{14}C\) into D-apiose units of cell wall polysaccharide
when Lemna was grown in presence of various
labeled precursors

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Amount administered</th>
<th>D-Apiose isolated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Weight ((\mu)C)</td>
<td>Activity ((\mu)C)</td>
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<tr>
<td>D-Glucose-1\textsuperscript{14}C</td>
<td>3.2</td>
<td>10</td>
</tr>
<tr>
<td>D-Glucose-2\textsuperscript{14}C</td>
<td>3.3</td>
<td>10</td>
</tr>
<tr>
<td>D-Glucose-3,4\textsuperscript{14}C</td>
<td>16.4</td>
<td>10</td>
</tr>
<tr>
<td>D-Glucose-6\textsuperscript{14}C</td>
<td>1.5</td>
<td>10</td>
</tr>
<tr>
<td>DL-Serine-3\textsuperscript{14}C</td>
<td>2.0</td>
<td>20</td>
</tr>
<tr>
<td>L-Methionine-l\textsuperscript{14}CH\textsubscript{3}</td>
<td>0.7</td>
<td>10</td>
</tr>
<tr>
<td>Sodium acetate-2\textsuperscript{14}C</td>
<td>22.2</td>
<td>100</td>
</tr>
</tbody>
</table>

Incorporation of Labeled Substrates into D-Apiose Units of
Lemna Cell Wall Polysaccharide In a series of experiments, Lemna was incubated in the presence of specifically labeled D-glucoses, DL-serine-3\textsuperscript{14}C, sodium acetate-2\textsuperscript{14}C, and L-methionine-\textsuperscript{14}CH\textsubscript{3}. After 7 days, D-apiose was isolated from the cell wall polysaccharide, and its specific activity was determined. Metabolism of D-glucose-1\textsuperscript{14}C, D-glucose-2\textsuperscript{14}C, and D-glucose-3,4\textsuperscript{14}C by Lemna resulted in the formation of D-apiose with a high specific activity, in contrast to D-glucose-6\textsuperscript{14}C, which was only about one-third as effective (Table II). These results are in agreement with those found for the biosynthesis of D-apiose in parsley plants (6), and suggest that D-apiose is derived directly from D-glucose. Although more D-glucose-3,4\textsuperscript{14}C was fed (10.4 \(\mu\)moles compared to 3.2 and 3.3 \(\mu\)moles for D-glucose-1\textsuperscript{14}C and D-glucose-2\textsuperscript{14}C, respectively), the specific activity of the isolated D-apiose was similar in all three cases. L. minor contains large amounts of polysaccharide which equilibrate rapidly and continuously with the endogenous D-glucose pool. Since the rate of synthesis of D-apiose for the formation of cell wall polysaccharide, which is dependent on growth, is relatively slow compared to the rate of turnover of starch, sucrose, and other polysaccharides containing D-glucose, the contribution of small amounts of exogenous D-glucose carbon to the pool size will be very small. This may explain why the specific activity of D-apiose derived from D-glucose-3,4\textsuperscript{14}C was similar to that obtained with D-glucose-1\textsuperscript{14}C and D-glucose-2\textsuperscript{14}C, even though 5 times as much D-glucose-3,4\textsuperscript{14}C was used. When D-glucose-6\textsuperscript{14}C was fed, the isolated D-apiose contained only 4000 dpm per \(\mu\)mole, and most of this radioactivity was located in C-1 of D-apiose (Table III). A similar effect was also observed with DL-serine-3\textsuperscript{14}C, further indicating that a significant holdup and turnover of D-glucose occur during the period of synthesis of D-apiose in this tissue. Of the possible 1-carbon donors tested, DL-serine-3\textsuperscript{14}C gave D-apiose of high specific activity, whereas L-methionine-\textsuperscript{14}CH\textsubscript{3} and acetate-2\textsuperscript{14}C were much less effective (Table II).

Distribution of \(^{14}C\) in D-Apiose—The purified D-apiose synthesized from each labeled substrate was diluted with unlabeled carrier. Half of the sample was reduced with sodium borohydrute to D-apiitol. The D-apiose and D-apiitol were oxidized with sodium metaperiodate to formaldehyde, formic acid, and glycolic acid. These products were isolated, the glycolic acid was oxidized to carbon dioxide and formic acid, and the specific activity of all the products was measured. The specific activity of C-1 of D-apiose was calculated by subtracting the specific activity of C-2, obtained as formic acid from the degradation of D-apiose, from the specific activity of C-1 plus C-2 obtained as formic acid from the degradation of D-apiose, as shown in Fig. 3. The activity of C-1 was also determined from the difference between the specific activity of C-1 plus C-4, obtained as formaldehyde, and the specific activity of the hydroxymethyl group of the glycolic acid (C-4) formed by oxidation of D-apiitol with periodate.

The results summarized in Table III show that, when D-glucose-1\textsuperscript{14}C was fed, 63% of the \(^{14}C\) incorporated into D-apiose was located at C-1. The other carbon atoms of D-apiose also contained small amounts of \(^{14}C\). Some randomization of the \(^{14}C\) always occurred after administration of the labeled D-glucoses.

The D-apiose derived from D-glucose-2\textsuperscript{14}C contained 51% of the incorporated \(^{14}C\) at C-2 and 16.6% at C-4, whereas the other carbon atoms contained somewhat smaller amounts of \(^{14}C\). The randomization of D-glucose-2\textsuperscript{14}C by the Embden-Meyerhof pathway would be expected to introduce \(^{14}C\) predominantly into C-5 of D-glucose. The activity found in C-4 of D-apiose can be accounted for by 45% randomization of D-glucose-2\textsuperscript{14}C, followed by the conversion of C-5 of D-glucose to C-4 of D-apiose. When D-glucose-3,4\textsuperscript{14}C was fed, C-3 of D-apiose contained 43.5% of the \(^{14}C\) incorporated, and C-4 and C-4 contained 25.2% and 13.2%, respectively. Thus, it would appear that both C-3 and C-4 of D-glucose are also utilized in the synthesis of D-apiose.

It should also be noted that the oxidation of the D-apiose derived from either D-glucose-2\textsuperscript{14}C or D-glucose-3,4\textsuperscript{14}C resulted in an unequal distribution of \(^{14}C\) between the two hydroxymethyl groups. This result suggests that the two groups are derived from different carbon atoms of D-glucose, and that the periodate ion differentiates between the two hydroxymethyl groups in the oxidation reaction. This effect was not observed when D-apiitol was oxidized, since the periodate ion cannot react preferentially with one of the hydroxymethyl groups when the carbon chain is in the acyclic form (Fig. 3).

The amount of formaldehyde derived from C-4 and C-4' of D-apiose will be different if periodate reacts more rapidly with the cis-diol (D-apio-D-furanose) form than with the trans-diol (D-apio-L-furanose) form (38). Isotopic randomization of D-glucose-2\textsuperscript{14}C may yield D-apiose labeled in position C-4, whereas D-glucose-
Table III

Distribution of $^{14}$C in isolated d-apiose moiety of cell wall polysaccharide after administration of labeled compounds to L. minor

<table>
<thead>
<tr>
<th>Precursor</th>
<th>Carbon atoms of d-apiose</th>
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<tr>
<td></td>
<td>C-1$^a$</td>
</tr>
<tr>
<td>d-Glucose-1-$^{14}$C</td>
<td></td>
</tr>
<tr>
<td>Specific activity (dpm/µmole)</td>
<td>840</td>
</tr>
<tr>
<td>Per cent of activity of d-apiose</td>
<td>100</td>
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<tr>
<td>n-Glucose-2-$^{14}$C</td>
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</tr>
<tr>
<td>Specific activity (dpm/µmole)</td>
<td>940</td>
</tr>
<tr>
<td>Per cent of activity of d-apiose</td>
<td>100</td>
</tr>
<tr>
<td>n-Glucose-3,4-$^{14}$C</td>
<td></td>
</tr>
<tr>
<td>Specific activity (dpm/µmole)</td>
<td>1150</td>
</tr>
<tr>
<td>Per cent of activity of d-apiose</td>
<td>100</td>
</tr>
<tr>
<td>d-Glucose-6-$^{14}$C</td>
<td></td>
</tr>
<tr>
<td>Specific activity (dpm/µmole)</td>
<td>455</td>
</tr>
<tr>
<td>Per cent of activity of d-apiose</td>
<td>100</td>
</tr>
<tr>
<td>nL-Serine-3-$^{14}$C</td>
<td></td>
</tr>
<tr>
<td>Specific activity (dpm/µmole)</td>
<td>810</td>
</tr>
<tr>
<td>Per cent of activity of d-apiose</td>
<td>100</td>
</tr>
</tbody>
</table>

* Value calculated from the specific activity of C-1 + C-2 minus the specific activity of C-2 obtained from the degradation of d-apiole.

" Value obtained by degradation of d-apiole.

 Obtained from the hydroxymethyl group of glycosidic acid.

 Obtained as formaldehyde.

3,4-$^{14}$C labels the C-4' group (Fig. 3 and Table III). Thus, if only one of the hydroxymethyl groups contains $^{14}$C, the ratio of $^{14}$C found in the C-4 compared to the C-4' group after equilibration between the two ring forms should vary inversely in these two experiments. The results obtained in the experiments shown in Table III are consistent with this proposal, since the ratio of $^{14}$C in the two hydroxymethyl groups of d-apiole varied from approximately 2.0 to 0.5 (16.6/8.5 compared to 13.2/25.2) with d-glucose-2-$^{14}$C and d-glucose-3,4-$^{14}$C, respectively.

Less $^{14}$C was incorporated into d-apiole when d-glucose-6-$^{14}$C was administered to L. minor, even though its specific activity was higher than any of the other labeled d-glucoses fed (Table II). Moreover, 48.5% of the $^{14}$C incorporated was present in C-1 of d-apiole (Table III). Randomization of n-glucose-6-$^{14}$C by enzymes of the Embden-Meyerhof pathway would result in a transfer of about 20 to 30% of the activity to C-1, which would account for the incorporation observed with this substrate after the loss of C-6. DL-Serine-3-$^{14}$C was also an effective precursor of d-apiole. The distribution pattern of $^{14}$C in d-apiole derived from DL-serine was almost identical with the pattern obtained with d-glucose-1-$^{14}$C. It would appear that d-glucose is an intermediate in the conversion of L-serine to d-apiole.

It has been suggested that streptose (3-C-formyl-5-deoxy-L-lyxose) is synthesized by the transannular rearrangement of a 4-hexosulose nucleoside 5'-pyrophosphate (36). A similar sequence of reactions has been postulated to account for the conversion of d-glucose into d-apiole (7, 37, 38). A reaction sequence involving a transannular rearrangement in three stages—an aldol cleavage, isomerization, and an intramolecular aldol condensation, which introduces the branched structure—is illustrated in Fig. 4. The results obtained in the present study are consistent with this mechanism, in that d-apiole is derived from carbon atoms 1 through 5 of d-glucose. C-1 and C-2 of d-apiole are derived from C-1 and C-2 of d-glucose, respectively; C-3 and C-4 of d-apiole are derived from C-3 and C-4 of d-glucose; and C-4 of d-apiole is derived from C-5 of d-glucose.

References

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