Steric Specificity in Synthesis of Heptuloses by Plants

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Of the possible stereoisomers of heptuloses, only one, d-altro-heptulose (sedoheptulose), has been assigned a specific role in plant metabolism (1, 3). Enzymic studies have revealed that phosphate esters of d-altro-heptulose can be produced in vitro in the presence of enzymes which are known to occur in plant tissues (5). That the heptulose can be metabolized in plant leaves has also been shown (2, 26).

Heptuloses other than d-altro-heptulose have been found in plants. d-manno-heptulose has been identified in a variety of species (11, 15, 17, 22), and talo-heptulose tentatively has been identified in avocado fruits (6). Heptuloses have been induced to accumulate in plant tissues by introducing into the tissues sugars which presumably serve as precursors. When the pentose d-ribose was fed to plant tissues d-altro-heptulose accumulated, L-arabinose on feeding gave L-gluco-heptulose, d-xyllose gave d-ido-heptulose, and L-lyxose gave L-galacto-heptulose (fig 1) (21). The hexulose L-sorbose also gave rise to L-galacto-heptulose (13). The 4 pentoses have the common feature that the -OH group on C-2 is in the d position (on the right hand side of the chain when shown in Fischer planar projection). This configuration is preserved in the derived heptuloses which exhibit d-threo configurations at C-3 and C-4. The 4 enantiomeric pentoses, with the -OH group on C-2 in the L position, do not cause heptuloses to accumulate.

The purpose of the present study was to determine whether any of the four tetrooses, or hexuloses other than L-sorbose, would cause heptulose accumulation when introduced into plant tissue. If accumulation takes place, would the heptuloses produced likewise have the d-threo configuration at C-3 and C-4? If the tetroses are incorporated with their carbon chains intact into heptuloses and if d-threo configurations are produced then (fig 1) d-erythrose would give d-altro-heptulose, L-erythrose would give L-gluco-heptulose.
The hexulose-heptulose interconversion must take into account reactions in which C-C bonds are split, and an intermediate compound thereby formed, as well as those reactions in which carbons become linked. The D-fructose-D-erythrose-D-allo-heptulose relationship exemplifies this type of reaction. If proper configuration were of concern only for the linkage reaction, D-tagatose should serve as well as L-sorbose, and D-allulose as well as D-fructose, for inducing heptulose biosynthesis. That D-fructose is not the only hexulose which can be converted to a tetrose is indicated by the recent finding in chromatographic studies that threose is present in plant tissue into which L-sorbose has been introduced (V. V. Rendig and E. A. McComb, unpublished).

Materials and Methods

Preparation of Sugars. Both L- and D-threose were prepared by the Wohl degradation of L- and D-xylose, respectively, according to methods described by Maquenne (12) and Hockett (8). The chromatographic behavior of the products was compared with a sample of L-threose obtained from Dr. A. S. Perlin. In our systems, and as consistently observed with other sugars, the L and D forms have identical chromatographic properties.

L-erythrose and D-erythrose were prepared from L- and D-arabinose, respectively, by the modification of the Wohl degradation referred to above. Another sample of D-erythrose was prepared by hydrolyzing (23) a sample of 2,4-O-ethylidene-D-erythrose obtained from Dr. H. S. Isbell. The resulting product was tested and found to be chromatographically pure.

One sample of D-tagatose was obtained from L. Light and Co., Ltd., Colnbrook, Bucks, England. Another sample was prepared by isomerization of D-galactose. and was purified by chromatographic techniques. D-sorbose and L-tagatose also were prepared by this procedure from D-galactose and L-sorbose, respectively.

Chromatography. Descending chromatography was used with Whatman No. 1 paper and with the following irrigants: A, ethyl acetate-pyridine-water (8: 2: 1 v/v) and B, phenol-water (10: 2 v/v). When 2-dimensional chromatography was used, irrigant A preceded irrigant B. For developing chromatograms, orcinol, aniline, or silver nitrate reagents were used (22). Under the conditions of this study the orcinol-trichloroacetic acid reagent produced a typical blue color (blue-green for manno-heptulose).

Since manno-heptulose occurs naturally in alfalfa (22) and since this sugar is available in crystalline form, it was used as a chromatographic reference standard. To determine the distance which it traveled, a sample of a 2% solution of manno-heptulose was superimposed on the same spot on the paper where a sample of plant extract had been applied previously and allowed to dry. The paper then was irrigated in the usual way.

Feeding of Sugars. Fully developed leaves from 2 to 3 week old alfalfa plants were used for all of the tests. The sugars were introduced into the tissue by immersing the petioles, with blades attached, into a 0.05 or 0.1 M solution of the sugar being tested. Preliminary tests had revealed that there was no significant difference in the results obtained using these 2 concentrations. Similar leaves with petioles immersed in distilled water were used as controls. All leaves were left in a lighted (250-350 ft-c) laboratory hood at a temperature of about 25° for 5-6 hours, after which the blades were excised and the juice expressed as previously described (21).

Results

Table I shows the results obtained by 1-dimensional chromatography of extracts from leaves into

<table>
<thead>
<tr>
<th>Sugar fed</th>
<th>R_manno-heptulose * of unknown orcinol complex</th>
<th>Known sugar used for co-chromatography</th>
<th>R_manno-heptulose of known sugar- orcinol complex</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-threose</td>
<td>0.81</td>
<td>L-galacto-heptulose**</td>
<td>0.80</td>
</tr>
<tr>
<td>L-sorbose</td>
<td>0.80</td>
<td>L-galacto-heptulose**</td>
<td>0.80</td>
</tr>
<tr>
<td>L-erythrose</td>
<td>0.95</td>
<td>L-gluco-heptulose**</td>
<td>0.94</td>
</tr>
<tr>
<td>D-erythrose</td>
<td>1.38</td>
<td>D-allo-heptulose***</td>
<td>1.38</td>
</tr>
<tr>
<td>D-threose</td>
<td>1.63</td>
<td>D-idu-heptulose†</td>
<td>1.62</td>
</tr>
</tbody>
</table>

\* R_manno-heptulose = distance of unknown or known sugar from origin.

** Authentic samples.

*** Preparation from Sedum conatum.

† Present in extract from leaves fed D-xylose.
which the various sugars had been introduced. The Rmanno-heptulose values for the heptuloses whose chromatographic behavior indicated a similarity to the unknown are also tabulated.

Feeding D-erythrose. The Rmanno-heptulose of the only orcinol-reactive compound which was present in leaves fed D-erythrose and not in those kept in water, was identical to that of D-altro-heptulose (Table 1). The heptulose from the D-erythrose fed tissue also cochromatographed with D-altro-heptulose from Sedum confusum. Judging from the intensity of the color formed in the orcinol reaction, the amount of D-altro-heptulose accumulating in the tissue was the least of all the heptuloses found by feeding the various sugars.

Feeding L-erythrose. The Rmanno-heptulose of the orcinol-reactive compound present in leaves fed L-erythrose was almost identical to that of L-gluco-heptulose (Table 1). In tests using 2-dimensional chromatography L-galacto-heptulose was superimposed on the extract from leaves fed L-erythrose. Clear separation of the 2 areas which formed orcinol-reactive blue products was obtained. The compound present in the leaves fed L-erythrose traveled further than did L-galacto-heptulose in irrigant A, but not as far in irrigant B as the latter heptulose. This is in accord with the expected behavior of L-gluco-heptulose.

Feeding D-threose. Characterization by 1-dimensional chromatography of an orcinol-reactive compound present in alfalfa leaves into which D-threose had been introduced indicated the compound to be D-idol-heptulose. The Rmanno-heptulose values of this heptulose and of the unknown were 1.62 and 1.63, respectively (Table 1). Further evidence was obtained by 2-dimensional chromatography. On one chromatogram an extract from leaves fed D-threose, and a D-altro-heptulose preparation from Sedum confusum, were superimposed at the origin; on another, these two, plus an extract from leaves into which D-xylose had been introduced, were superimposed. After irrigation and orcinol treatment 2 blue areas appeared on both chromatograms. The intensity of the color of the D-altro-heptulose area was the same on both; the color intensity of the other area was increased on the chromatogram on which the extract from the tissue fed D-xylose had been included. In earlier studies the product formed in tissue fed D-xylose had been identified by means of x-ray diffraction pattern and rotation as D-idol-heptulose (21).

Feeding L-threose. Chromatography of leaf extracts indicated that L-threose, like L-sorbose, induced an accumulation of L-galacto-heptulose. The heptulose which, because of similar chromatographic behavior, could be most easily confused with L-galacto-heptulose is L-gluco-heptulose. That this latter heptulose was not the one in the tissue into which L-threose was introduced was shown by 2-dimensional chromatography. While in irrigant A the unknown heptulose in the leaves fed L-threose traveled further than did L-gluco-heptulose, in irrigant B, the relative rates were reversed. This is in accord with our previous observations and the report of Noggle (16). Two-dimensional chromatography also indicated the presence of sorbose in extracts from leaves fed L-threose. The Rmanno-heptulose values of sorbose and fructose in irrigant A are identical, but in irrigant B fructose separates from sorbose. Treatment with silver nitrate reagent of the chromatograms on which had been applied extracts from leaves into which L-threose had been introduced revealed the presence of a compound whose Rmanno-heptulose value was identical to that of threitol. L-threitol has been isolated and identified in the leaves of alfalfa into which L-sorbose has been introduced (14).

Feeding Hexuloses. The chromatographic evidence for the presence of L-galacto-heptulose in leaves fed L-sorbose is shown in Table 1. The identity of this heptulose was established previously (13) by its x-ray powder diffraction pattern and its rotation. Unlike L-sorbose, D-sorbose did not cause any heptulose to accumulate to a chromatographically detectable level.

No chromatographically detectable amounts of any heptulose accumulated in plant tissue into which D- or L-tagatose or D-fructose was introduced. All of these hexuloses were taken into leaves readily as evidence by positive chromatographic tests for these hexuloses in extracts from leaves fed the respective sugar. No difference in behavior of the D-tagatose obtained from the 2 sources was indicated by the results.

Discussion

Figure 1 shows the molecular configuration of the heptuloses which were found in alfalfa leaves into which one or another of the 4 tetroses had been introduced. The 4 pentoses which in previous studies (21) had been found to induce these 4 heptuloses to accumulate are shown also. L-lyxose, D-lyxose, and D-arabinose did not cause any heptulose to accumulate to chromatographically detectable levels.

That plants do contain enzymes which are able to catalyze the conversion of tetroses directly to heptuloses has been demonstrated in other studies. One possibility is an aldolase-catalyzed condensation of the tetrose with dihydroxyacetone phosphate. The same tetrose-heptulose conversions indicated in the present study have been demonstrated in vitro by Jones and co-workers (7, 10). Aldolase used in their studies was prepared from peas and fructose 1,6-diphosphate was provided as a donor of triose phosphate. The tetrose was added to the incubation mixture as the free sugar not as the phosphate. In the present studies direct evidence was not obtained for a likely source of a triose to attach to the tetrose by the reactions postulated in these enzymic studies. Certainly there was not sufficient endogenous dihydroxyacetone phosphate or hexose diphosphate present in the leaves at the start of the test but more could be produced at the expense of ATP initially present or formed during the time the sugars were being fed.
Another possibility is a linkage of carbon 1 of the tetrose to transaldolase-activated dihydroxyacetone (9, 27). The only compound which was detected in the leaves used in the present studies and which is known to serve as a dihydroxyacetone donor (4) is d-fructose. Other compounds which have been shown to serve in vitro as donors are d-altro-heptulose-7-P (9), erythrulose (27), L-sorbose-6-P (27) and octulose-8-P (19). Of these, d-altro-heptulose-7-P would most likely be present but octuloses also have been identified in alfalfa leaves (22). If the conversion of tetrose to heptulose is via a transaldolase-mediated reaction the tetrose would likely first have to be phosphorylated. The reason that tetrose phosphate was not observed in the alfalfa leaves could be that it had a transitory existence and never accumulated sufficiently to be detected chromatographically.

Steric considerations can also be invoked to explain in part why no evidence was revealed for products of transketolase activity which had been shown to be considerable in alfalfa leaves in previous studies (21). The -OH group on carbon 2 in d-threose and L-erythrose is not correctly oriented to yield in a transketolase-catalyzed reaction a hexulose having the d-xylulose configuration on carbons 3 and 4 (18). The inertness of the d-tagatose and d-sorbose introduced into leaves in the present studies is consistent with this line of reasoning. If steric considerations did not apply these 2 hexuloses would be the predicted products of a transketolase-catalyzed reaction in which d-threose served as substrate. L-threose as a substrate in a transketolase-catalyzed reaction would

Fig. 1. Heptuloses formed in leaf tissue into which the indicated tetrose or pentose was introduced.
yield a product having the d-xylulose configuration, namely, L-sorbose. The feeding of this hexulose to alfalfa leaves results in accumulation of a hexulose which was identified in previous studies (13) as L-galacto-heptulose, the same hexulose which the present studies has shown to result from L-threose feeding. As previously mentioned, direct evidence for the formation of L-threose from L-sorbose has been obtained. d-erythrose would likewise yield a product in a transketolase-catalyzed reaction which would have the d-xylulose configuration, namely d-fructose. Considering the great number of reactions by which this hexulose can be metabolized it is not too surprising that heptulose could not be detected in leaves into which it was introduced. This also may explain why the amount of d-altro-heptulose in leaves fed d-erythrose appeared to be less than the amount of heptulose formed from any other tetrose.

The findings of the present study offer no clue to the route of synthesis of the ubiquitous d-manno-heptulose which has the L-erythro configuration at carbons 3 and 4. In none of our studies was the abundance of this heptulose altered sufficiently to attribute its presence to the action of an epimerase.

Both the threo and erythro configurations are represented in the naturally occurring monosaccharides having more than 7 carbon atoms. The d-erythro-L-glucu-nonulose (24) and the d-glycero-L-galacto-octulose (25) isolated recently from the avocado have the d-threo configuration, but the d-glycero-d-manno-octulose isolated from the same species and from Sedum (6) and alfalfa (22) has the L-erythro configuration.

Summary

All of the 4 tetrose stereoisomers and several hexuloses were introduced separately into alfalfa leaves and, after a 5 to 6 hour period, extracts from the leaves were examined chromatographically for the presence of heptuloses which were then subjected to further tests for identification.

Each of the 4 tetroses induced an accumulation of a specific heptulose, each having the d-threo configuration at C-3,4. d-erythrose gave d-altro-heptulose, L-erythrose gave L-glucu-heptulose, d-threose gave d-idu-heptulose, and L-threose gave L-galacto-heptulose. In being converted the tetrose molecule appears to remain intact since its configuration at C-2,3 is preserved as C-5,6 in the heptulose molecule which it induces. Although no direct evidence for such intermediates was obtained, phosphorylation of the tetrose or of the group with which it condenses probably occurs whether the reaction involved is aldolase- or transaldolase-catalyzed. The suitability of hexuloses for heptulose biosynthesis in alfalfa leaves appears to be contingent upon their having the C-3,4 configuration characteristic of transketolase substrates. d- and L-tagatose and d-sorbose which have the L-erythro, d-erythro and L-threo configuration, respectively, on C-3,4 did not cause any heptulose to accumulate, while L-sorbose, which has the d-threo configuration, induced the accumulation of L-galacto-heptulose. That d-fructose, which also has the d-threo configuration and has been shown to be convertible to d-altro-heptulose in vitro, failed to induce heptulose accumulation is attributed to its serving as substrate for other competing metabolic reactions. This interpretation would be consistent with our observation that d-erythrose, which would be formed from d-fructose by transketolase, induced much less accumulation of heptulose than did d- or L-threose. The latter would be the transketolase-catalyzed reaction products of the three hexuloses found to be inert in inducing heptulose synthesis.

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Literature Cited


