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# BIOSYNTHESIS OF D- AND L-*glycero*-L-*galacto*-OCTULOSE FROM PENTOSES AND HEXOSES*

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ABSTRACT

D-*glycero*-L-*galacto*-Octulose and L-*glycero*-L-*galacto*-octulose accumulated when leaves of Kenland red clover (*Trifolium pratense*) were allowed to imbibe solutions of D-gulose or D-xylose and L-mannose or L-arabinose, respectively. The octuloses were isolated and identified by paper chromatography and by oxidative degradations to the corresponding lower sugars. Assignments of the D and L configuration were made on the basis of optical rotation. It is suggested that formation of the octuloses from the hexoses and pentoses is mediated through transketolase and aldolase or transaldolase catalysis, respectively.

## INTRODUCTION

Octuloses occur naturally in a number of higher plants. D-*glycero*-D-*manno*-Octulose appears to be ubiquitous[1–4], whereas D-*glycero*-L-*galacto*-octulose has been identified in only a few species, including *Persea gratissima*[5], *Sedum spectabile*[6], and *Primula officinalis*[1]. Jones and Sephton[7] prepared four octuloses having the D-*threo* configuration at C-3 and C-4 by aldolase catalysis, using D-fructose 1,6-diphosphate and the corresponding pentoses as substrates. Our interest in the routes whereby these ketoses are formed *in vivo* led to the present study.

The specific purpose of our work was to show whether the systems operative for the formation of heptuloses in plant leaves also apply to the formation of octuloses. Previous studies[8,9] revealed that heptuloses accumulate in the leaves of several higher plants which have imbibed solutions of pentoses having the D configuration at C-2. For each of these pentoses, a particular heptulose having the D-*threo* configuration at C-3 and C-4 is formed. The configuration about C-5 and C-6 of the heptulose formed is the same as that of the pentose supplied. Heptulose formation has been ascribed to transketolase (EC 2.2.1.1) catalysis[9]. It has also been shown[10] that when leaves imbibe any of the four tetroses, heptuloses having a D-*threo* configuration at C-3 and

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C-4 accumulate. The configuration about C-5 and C-6 of the heptuloses is the same as that of the tetrose supplied. The enzymes transaldolase (EC 2.2.1.2) or aldolase (EC 4.1.2.13) were suggested as possible catalysts for this reaction.

If the principles just cited apply, an octulose having the D-*threo* configuration at C-3 and C-4 should be formed when any hexose having the D configuration at C-2 is introduced. The configuration at C-2, C-3, C-4, and C-5 of the hexose should be retained in the octulose. Likewise, from any pentose, an octulose should be synthesized having the D-*threo* configuration at C-3 and C-4 and having the same configuration about C-5, C-6, and C-7 as that of the pentose (Fig. 1).

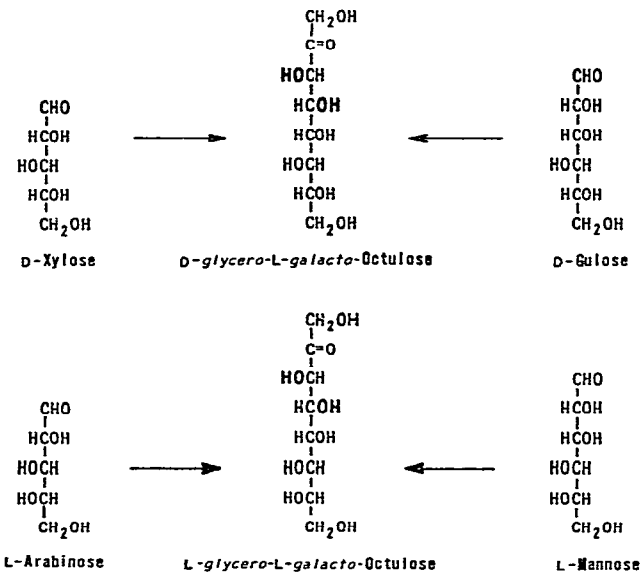


Fig. 1. Octulose formation from pentoses and hexoses.

## RESULTS AND DISCUSSION

Two-dimensional paper chromatography (irrigants *A* and *B*) of juice expressed from leaves that had imbibed D-gulose and D-xylose revealed the presence of a compound suspected to be D-*glycero*-L-*galacto*-octulose. It co-chromatographed with D-*glycero*-L-*galacto*-octulose and, after development with orcinol[11], showed a pink, fading-to-gray spot characteristic of octuloses.

Using a similar procedure, the presence of L-*glycero*-L-*galacto*-octulose was indicated in extracts from leaves supplied with L-mannose and L-arabinose. In contrast, there was no indication of octulose formation from D-mannose.

After extraction and purification of the octulose preparations, the yield was 30–40 mg (syrups) in each case, but the preparations were contaminated by compounds that reacted with silver nitrate.

Confirmatory evidence for the identification of the suspected octuloses was obtained by using oxidative degradation procedures. Oxidation with lead tetraacetate[12] gave xylose as the main product from the compound which chromatographic evidence had indicated to be D-*glycero*-L-*galacto*-octulose. Likewise, as predicted, arabinose was the oxidation product from the sugar that chromatographed with L-*glycero*-L-*galacto*-octulose. This suggests that the former octulose has the *xylo* and the latter the *arabino* configuration at C-5, C-6, and C-7. Periodate oxidation[12] and subsequent reduction and hydrolysis of the methyl octulosides[13] yielded *galacto*-heptulose as the only orcinol-reactive product, indicating that both octuloses have the *galacto* configuration from C-3 through C-7.

D-*glycero*-L-*galacto*-Octulose formed from D-gulose had $[\alpha]_D$ −21° (*c* 1.8), and the octulose formed from D-xylose had $[\alpha]_D$ −28° (*c* 1.3). The literature $[\alpha]_D$ values for D-*glycero*-L-*galacto*-octulose are −57° and −61° (ref. 5), and −43.4° → −13.4° (ref. 7). L-*glycero*-L-*galacto*-Octulose formed from L-mannose had $[\alpha]_D$ −55° (*c* 0.47), and the octulose from L-arabinose had $[\alpha]_D$ −68° (*c* 0.77); a value of −62° has been reported[7] for this compound. The discrepancies of our values, as compared with each other and with the ones previously reported, might be explained by the fact that our preparations were all contaminated. Both our values and those previously reported were obtained from syrupy samples. However, we believe that the signs of rotation, in comparison with those reported, are indicative of whether our octuloses should be assigned the D or L configurations.

When D-gulose, D-xylose, L-mannose, and L-arabinose were treated with yeast in the presence of D-fructose, under the same conditions as the plant extract, there was no evidence of octulose formation. Thus, the possibility that octuloses resulting from the fermentation procedure are contributing to our octulose preparations can be excluded.

Non-fermentable, free carbohydrates of Kenland red clover have been investigated by McComb (unpublished results). D-*glycero*-D-*manno*-Octulose was the only octulose to be detected. Xylose and arabinose were also found to be present. Bonner[14] included D-xylose and L-arabinose in a list of sugars commonly found in plants.

Begbie and Richtmyer[1] reported the occurrence of D-*glycero*-L-*galacto*-octulose, together with D-xylose, in *Primula officinalis*. Knowing that octuloses are formed from pentoses by aldolase[7] and transaldolase[15] catalysis, one might expect to find the octuloses corresponding to xylose and arabinose as naturally occurring carbohydrates in clover. The failure to detect these octuloses might be explained by the small amounts of pentoses present. In contrast to the pentoses, the two hexoses used in the present study, D-gulose and L-mannose, have not been reported in higher plants.

Rendig and McComb[10] ascribed the formation of heptuloses from tetroses to transaldolase or aldolase catalysis. It might also be suggested that either transaldolase or aldolase is the enzyme catalyzing the formation of D-*glycero*-L-*galacto*-octulose and L-*glycero*-L-*galacto*-octulose from D-xylose and L-arabinose, respectively. Williams and Clark[16] have suggested that mono- and di-phosphates of D-*glycero*-

D-*ido*-octulose are intermediates in the pentose phosphate cycle, the diphosphates being formed from 1,3-dihydroxy-2-propanone phosphate and D-arabinose 5-phosphate by aldolase catalysis.

The detection of D-*glycero*-L-*galacto*-octulose and L-*glycero*-L-*galacto*-octulose in plants fed with D-gulose and L-mannose, respectively, is in accordance with transketolase catalysis, as suggested by Rendig and McComb[9] for heptulose formation from pentoses. Recently, Villafranca and Axelrod[17] demonstrated that neither the glycolyl donor nor its acceptor need to be phosphorylated in transketolase catalysis. It is reasonable to assume that transketolase also catalyzes the hexose–octulose conversion.

The requirement that the acceptor molecule should have the D configuration at C-2 is fulfilled with D-gulose and L-mannose. D-Mannose, having the L configuration at C-2, did not form an octulose. Both aldolase, transaldolase, and transketolase are known to form aldol condensation products having the D-*threo* configuration at C-3 and C-4, as is the case with D-*glycero*-L-*galacto*-octulose and L-*glycero*-L-*galacto*-octulose.

The results of this study indicate a pathway by which octuloses having the D-*threo* configuration at C-3 and C-4 can be formed in higher plants. They do not, however, offer any clue to the kind of metabolic reaction by which the commonly occurring D-*glycero*-D-*manno*-octulose, having the L-*erythro* configuration at C-3 and C-4, is synthesized.

## EXPERIMENTAL

*General.* — Whatman No. 1 paper was used for analytical chromatography, and Whatman No. 3MM for isolation purposes. The irrigants used were (*A*) ethyl acetate–pyridine–water (8:2:1), (*B*) liquified phenol–water (10:2), (*C*) ethyl acetate–acetic acid–water (9:2:2), and (*D*) 1-butanol–ethanol–water (3:1:1). Chromatograms were developed with orcinol[11], aniline[18], and silver nitrate[19]. Optical rotations were measured in water.

D-Gulose was prepared from D-gulono-1,4-lactone, essentially as described by Isbell[20]. The preparation was purified on heavy paper, using irrigant *C*. L-Mannose was synthesized directly[21] from L-arabinose, and was also obtained from Pfanstiehl Laboratories. D-*glycero*-L-*galacto*-Octulose and L-*glycero*-L-*galacto*-octulose were prepared enzymically[7].

*Feeding of sugars to plant leaves.* — Fully developed leaves of Kenland red clover (*Trifolium pratense*) grown in a growth chamber (day temperature 24°, night temperature 22°; 12 h light, 1500 ft-c, and 12 h dark) were used in the experiments. The leaves were allowed to imbibe ~0.1M solutions of D-xylose, L-arabinose, D-gulose, and L-mannose. About 1000 leaves were used for each preparation. The pentoses were supplied in the dark for ~20 h, and the hexoses for 8 h under 1500 ft-c illumination. Juice was extracted from the fresh leaves according to the procedure of McComb and Rendig[22].

For characterization purposes, extractions and purifications were done as described by McComb and Rendig[23]. A fermentation step was included[24]. After filtration and deionization, the mixtures containing the respective octuloses were chromatographed on heavy paper in system *A*. The mixture resulting from the feeding of L-arabinose was also chromatographed in system B. The octuloses formed were eluted from the chromatogram with water. Optical rotation measurements were taken prior to degradation of the isolated sugars.

*Characterization of the octuloses.* — The octuloses were identified by paper chromatography (systems *A*, *B*, and *C*) in comparison with reference material, and by oxidative degradations to the corresponding lower sugars. Thus, each octulose (~15 μmoles) was treated with lead tetra-acetate[12] (30 μmoles) and hydrolyzed as previously described. The resulting pentoses were identified by paper chromatography (systems *A* and *B*). The octuloses (~15 μmoles) were further transformed[13] into their methyl pyranosides, which were then oxidized with equimolar amounts of aqueous sodium metaperiodate, reduced, and hydrolyzed as described[12], except that room temperature rather than 0° was used for the oxidations. The identity of the heptulose formed was established by paper chromatography (systems *A* and *B*).

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