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Note

Biosynthesis of D-*glycero*-D-*altro*-octulose from D-ribose and D-allose

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Recently, we reported[1] that D-*glycero*-L-*galacto*-octulose accumulated in leaves of Kenland red clover (*Trifolium pratense*) allowed to imbibe solutions of D-gulose or D-xylose. Similarly, when L-mannose or L-arabinose was taken up, L-*glycero*-L-*galacto*-octulose was formed[1]. Based on previous studies in our laboratory and on reports in the literature, we ascribe the reaction involving the conversion of pentoses to aldolase or transaldolase catalysis. The hexose–octulose conversion may be catalysed by transketolase.

We now report on the biosynthesis of D-*glycero*-D-*altro*-octulose in leaves that have imbibed D-ribose or D-allose.

It is reasonable to assume that the pathways by which D-*glycero*-D-*altro*-octulose is formed in our study are the same as those already suggested[1] for D-*glycero*-L-*galacto*-octulose and L-*glycero*-L-*galacto*-octulose in plants allowed to imbibe certain pentoses or hexoses.

Formation of D-*glycero*-D-*altro*-octulose in a reaction mixture containing aldolase, fructose 1,6-diphosphate, and ribose has been reported by Jones and Sephton[2]. Racker and Schroeder[3] demonstrated that an octulose phosphate, probably D-*glycero*-D-*altro*-octulose 8-phosphate, was formed after reaction of transaldolase with ribose 5-phosphate and fructose 6-phosphate. They further reported that the octulose phosphate, in the presence of transketolase and a glycolyl acceptor, gave rise to a hexose phosphate, possibly allose 6-phosphate. An octulose diphosphate, which might well be the ester of D-*glycero*-D-*altro*-octulose, has been reported[4] as a normal constituent of red cells, its mono- and di-phosphates have been isolated from erythrocytes incubated with inosine[5]. Jones and Sephton[2] suggested that D-*glycero*-D-*altro*-octulose might be a precursor of the commonly found D-*glycero*-D-*manno*-octulose. This possibility could involve the action of a 4-epimerase. Such epimerases for other sugars have been shown to exist in lower organisms[6–8]. However, D-*glycero*-D-*altro*-octulose has not been found as a natural constituent in higher plants, even

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though one of its potential precursors, D-ribose, is ubiquitous It might be that this octulose is present only in a transient state, and thus has not been detected by the methods used.

In the present investigation, two-dimensional paper chromatography of juice expressed from leaves fed D-allose or D-ribose revealed the presence of a compound having the same chromatographic mobility as D-*glycero*-D-*altro*-octulose (R_{FRU} 0 89 0 65, and 0 63—in systems *A*, *B*, and *C*, respectively) Also, the color reaction with orcinol was characteristic for octuloses[1]

Amounts sufficient for the identification of the supposed octuloses were achieved through the purification of extracts from clover leaves allowed to imbibe either of the two potential precursors, D-allose and D-ribose, the yield was 6–8 mg (syrup) in each case Degradation with lead tetra-acetate of the two octulose preparations yielded ribose as the main oxidation product, indicating the presence of the *ribo* configuration at C-5, C-6, and C-7 in both cases Alkaline oxidation of the preparations with oxygen[2] gave, as one product, a compound having the same paper-chromatographic mobility and color reaction with hydroxamic acid[9] as did D-*glycero*-D-*altro* heptono-γ-lactone

Acid treatment of both octulose preparations gave rise to a compound having the same chromatographic mobility (fast-running) and the same color reaction with orcinol (intensely pink, visible without heating), as did an acid-treated reference sample of D-*glycero*-D-*altro*-octulose However, no attempt was made to establish the identity of this compound Jones and Sephton[2] reported the formation of a substance having similar chromatographic properties when the methyl glycoside of D-*glycero*-D-*altro*-octulose or the octulose 1-phosphate was treated with acid It has further been reported[10] that acid treatment of D-*altro*-heptulose (sedoheptulose) gives a product which is fast-running on paper chromatograms and gives a brilliant color with orcinol This compound was tentatively identified as 5-(1,2-dihydroxyethyl)-2-furaldehyde[11], and it is tempting to suggest that the compound derived from D-*glycero*-D-*altro*-octulose is a homologue of this substance Chromatographic mobilities, oxidative degradations, and the $[\alpha]_D$ values [+8° (*c* 0 25, water) for the octulose derived from D-ribose, and +5° (*c* 0 25, methanol) for the one originating from D-allose] indicate that our compound is D-*glycero*-D-*altro*-octulose Jones and Sephton reported $[\alpha]_D$ values of +8°, +8 4°, +7.9°, and +2 9° for this compound

The possibility that the octulose had been produced during the fermentation procedure was considered. No evidence of octulose formation was obtained, however, when D-allose and D-ribose were treated with yeast in the presence of D-fructose under the same conditions as the plant extracts.

EXPERIMENTAL

Whatman No 1 paper was used for analytical chromatography, and Whatman No 3MM paper for preparative purposes. The irrigants used were (*A*) ethyl acetate–pyridine–water (8 2 1), (*B*) liquefied phenol–water (10 2), and (*C*) 1-butanol–ethanol–

water (3 1.1) Detection was effected with orcinol[12], aniline[13], and silver nitrate[14] In addition, lactones were detected with a hydroxamic acid test[9] D-Allose was synthesised from D-ribose according to Humoller[15], and also obtained from P L Biochemicals D-*glycero*-D-*altro*-Octulose used for comparisons was prepared enzymically as described by Jones and Sephton[2]

Clover leaves were allowed to take up solutions of D-ribose (2000 leaves) and D-allose (1500 leaves) through the petioles under the conditions described in the previous paper[1] Juice was expressed from the fresh leaves according to the procedure of McComb and Rendig[16] A quantity of the unknown sugar was isolated[1] from an ethanolic extract of clover leaves After evaporation of the ethanol, the mixture was filtered through Celite, deionized by ionic exchangers, and fermented with bakers' yeast To obtain substances of sufficient purity, the compound derived from D-allose was purified by preparative paper chromatography in systems *A* and *C*, and the one obtained from D-ribose was also irrigated in system *B* Degradation with lead tetra-acetate of the octulose preparations (~ 10 μmoles) was performed as described[1] Alkaline degradation was performed on ~ 12 μmoles of each of the preparations, essentially as described by Jones and Sephton[2] The identity of the degradation products was established by paper chromatography

For acid conversion of the two preparations, ~ 10 μmoles of each were kept in 0 25M sulfuric acid at 100° for 16 h. The mixture was neutralized with barium carbonate, and the filtrate was deionized with ion-exchange resins

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REFERENCES

1 G Haustveit, E A McComb, and V V Rendig, *Carbohyd Res*, 39 (1975) 125
2 J K N Jones and H H Sephton, *Can J Chem*, 38 (1960) 753
3 E Racker and E Schroeder, *Arch Biochem Biophys*, 66 (1957) 241
4 B S Vanderheiden, *Biochem Biophys Res Commun*, 21 (1965) 265
5 G R Bartlett and G Buculo, *Biochim Biophys Acta*, 156 (1968) 240
6 D P Burma and B L Horecker, *J. Biol Chem*, 231 (1958) 1053
7 M J Wolin, F J Simpson, and W A Wood, *J Biol Chem*, 232 (1958) 559
8 N Lee, J W Patrick, and M Masson, *J Biol Chem*, 243 (1968) 4700
9 M Gee and R M McCready, *Anal Chem*, 29 (1957) 257
10 A Bevenue and K T Williams, *J Chromatogr*, 4 (1960) 391
11 L P Zill and N E Tolbert, *J Amer Chem Soc*, 76 (1954) 2929
12 R Klevstrand and A Nordal, *Acta Chem Scand*, 4 (1950) 1320
13 R M McCready and E A McComb, *Anal Chem*, 26 (1954) 1645
14 W E Trevelyan, D P Procter, and J S Harrison, *Nature (London)*, 166 (1950) 444
15 F L Humoller, *Methods Carbohyd Chem*, 1 (1962) 102
16 E A McComb and V V Rendig, *Chemist-Analyst*, 49 (1960) 55