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Note

Aldolase-catalysed formation of D-*glycero*-D-*manno*-octulose from D-ribose and D-fructose 1,6-diphospate

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Jones and Sephton[1] demonstrated the formation of four octuloses from pentoses and D-fructose 1,6-diphosphate by aldolase catalysis. The octuloses retained the configuration of the original pentoses at C-5,6,7 and had the D-*threo* configuration at C-3,4. The formation of octuloses from pentoses in plants has also been demonstrated[2,3], and it was suggested that either aldolose or transaldolase is the enzyme catalyzing the condensation.

On preparing D-*glycero*-D-*altro*-octulose from D-ribose and D-fructose by the method of Jones and Sephton[1], it was noticed that, in addition to the expected octulose, another compound was formed, which gave a colour with orcinol characteristic for octuloses[2]. This note describes the isolation and identification of the unknown octulose.

After treatment with phosphatase, fermentation, and preparative p.c., the octulose (3.7 mg), when isolated from an incubate of D-ribose, D-fructose 1,6-diphosphate, and aldolase (EC 4.1.2.13), had $[\alpha]_D$ +22° (*c* 0.2, water), and the same mobility in p.c. as D-*glycero*-D-*manno*-octulose (R_{FRU} 0.38 and 0.65, solvents *A* and *B*, respectively). Oxidation of the octulose with lead tetra-acetate[4] gave ribose, and periodate oxidation of its methyl glycosides followed by reduction and hydrolysis gave mainly *manno*-heptulose. Thus, the octulose has the *ribo* configuration at C-5,6,7, and the *manno* configuration at C-3,4,5,6. The yield of D-*glycero*-D-*manno*-octulose was ~10% of that obtained for D-*glycero*-D-*altro*-octulose from the same incubate.

In order to show that the D-*glycero*-D-*manno*-octulose was not formed in the other enzyme incubations, the phosphates from the aldolase incubation were purified on an anion-exchange resin[5], and then hydrolysed with acid. The products had chromatographic mobilities and colour reaction characteristic of D-*glycero*-D-*manno*-octulose and D-*glycero*-D-*altro*-octulose.

D-*glycero*-D-*manno*-Octulose is commonly found in higher plants[6–8] and might[1] be the product of the catalytic activity of a 4-epimerase on D-*glycero*-D-*altro*-

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octulose. Enzymes catalyzing C-4 epimerizations in ketoses have been described in lower organisms[9–11]. Apparently, D-*glycero*-D-*altro*-octulose has not been found in plants, but if present, for example as a phosphate, it would have been lost during deionization procedures.

D-*glycero*-D-*manno*-Octulose may also be a product of aldolase catalysis. Aldolase (EC 4.1.2.13) has been postulated[12] to give condensation products having the D-*threo* configuration at C-3,4. However, D-*glycero*-D-*manno*-octulose has the L-*erythro* configuration at these positions. Thus, its formation would imply an unspecific aldolase condensation taking place in addition to that expected. The cleavage of a ketose having a D-*erythro* configuration by rabbit muscle aldolase has been reported[13].

EXPERIMENTAL

General. — P.c. was effected on Whatman No. 1 and 3MM papers, using *A* ethyl acetate–pyridine–water (8:2:1), *B* phenol–water (10:2), and *C* butanone–acetic acid–water saturated with boric acid (80:10:10). Detection was effected with orcinol[14], aniline, and silver nitrate[15]. Borate complexes were detected with orcinol containing 10% of conc. hydrochloric acid. G.l.c. was performed on *O*-trimethylsilylated sugars[16], using a column (200 × 0.20 cm) of 3% of SE-52 on Gas-Chrom Q. Pentoses were analysed at 140° and heptuloses at 180° with a carrier-gas rate of 38 ml/min.

D-Ribose; D-fructose 1,6-diphosphate tetrasodium salt; aldolase from rabbit muscle (EC 4.1.2.13), grade 1; and acid phosphatase from wheat germ (EC 3.1.3.2) were obtained from Sigma.

Enzymic synthesis of the octuloses[1]. — An aqueous solution of D-ribose (1 g) and D-fructose 1,6-diphosphate tetrasodium salt (2 g) was incubated with 200 mg of aldolase (12 units/mg of protein). Incubation, subsequent dephosphorylation with phosphatase, and deionization were performed as described by Jones and Sephton[1]. The mixture was fermented[2] with bakers' yeast to remove the fructose, then filtered through Celite, and treated with ion-exchange resins. The concentrated mixture (340 mg) was fractionated by p.c. (solvent *A*). The zones corresponding to the two octuloses, detected by the orcinol test[2] on side strips, were eluted with water, and the solutions were deionized and concentrated. The slower-moving component was the unknown octulose.

Identification of the unknown octulose. — The octulose was identified by comparative p.c. (solvents *A* and *B*), optical rotation, and oxidative degradation with lead tetra-acetate, and by periodate oxidation of its methyl glycoside[4]. The identities of the resulting pentose and heptulose were established by g.l.c. and p.c. (solvents *A*, *B*, and *C*).

Isolation of the monophosphates. — D-Ribose (0.1 g) and the salt of D-fructose 1,6-diphosphate were incubated with aldolase as described above. After inactivation of the enzyme, the cooled filtrate was transferred to a column (1.5 × 22.5 cm) of Biorad AG-1 x4 ($HCOO^-$) resin (400 mesh). The neutral-sugar fraction was eluted

with water, and the monophosphate with a linear gradient (0→1M formic acid). Fractions (12 ml) were collected, and those (83–115) giving both positive octulose[17] and positive phosphate[18] tests were combined and concentrated.

The residue was treated with M hydrochloric acid at 100° for 2 h. After removal of hydrochloric acid by repeated evaporation with toluene and deionization, the neutral sugars were subjected to comparative p.c. (solvent *A*).

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