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Note

Preparation of D-sorbose from L-glucitol by bioconversion with *Pseudomonas* sp. Ac

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The ketohexose D-sorbose is a rare sugar which is of potential interest as a low-caloric sweetener [1,2], as an insect control agent [3], and as a starting material for producing industrially significant compounds such as L-*threo*-2,5-hexodiulose [4]. Chemical synthesis of D-sorbose has been accomplished from D-galactose [5,6] and from penta-*O*-acetyl-α-D-idopyranose [7] with yields of 11.4, 23–26, and 55–70%, respectively. Biotechnical synthesis of D-sorbose by microbial conversion of L-glucitol [4] and galactitol [8] resulted in yields of 60 and 70%, respectively, and only recently the preparation of D-sorbose from D-tagatose by an immobilized enzyme, D-tagatose-3-epimerase, with a yield of 70% has been reported [9].

In this communication we report the synthesis of D-sorbose from L-glucitol by microbial conversion with high yields (> 95%) and in a reasonable period of time. Unlike the previous report [4] in which complete conversion of L-glucitol into D-sorbose with an unidentified bacterial strain MD-13 required 5–6 days if the initial substrate concentration did not exceed 1%, the bioconversion described here was complete within 35 h, allowing substrate concentrations of up to 2.5%. Furthermore, L-glucitol (**2**) was not prepared by borohydride reduction from L-glucose [4] but from D-gulono-1,4-lactone (**1**) [10] which is a significantly cheaper starting material.

The regioselective oxidation at C-5 of **2** to give D-sorbose (**3**) (Scheme 1) is catalyzed by the enzyme L-glucitol dehydrogenase harboured by the newly isolated bacterium

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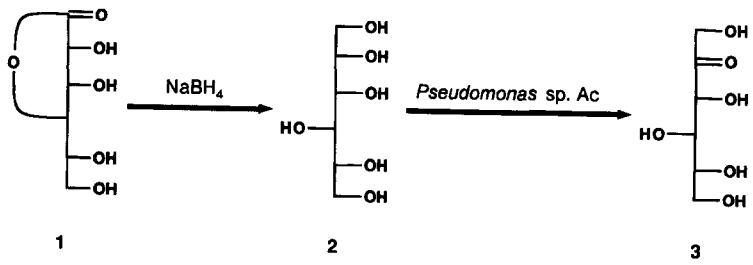

Scheme 1.

Pseudomonas sp. Ac [10]. Fig. 1 shows the time course of the bioconversion using washed cell suspensions of *Pseudomonas* sp. Ac in the presence of 120 mM **2** (4.37 g in 200 mL), to give **3** in yields of 97%. Then **3** was purified from the reaction solution by ligand exchange chromatography [10–12] and crystallized following evaporation. Crystalline **3** (4 g) was obtained, corresponding to an overall yield of 91%; **3** was identified by HPLC comparison with authentic D-sorbose, and by optical rotation, $[\alpha]_D^{20}$ +43° (*c* 1.0, water); {lit. [13] $[\alpha]_D^{23}$ +43° (water)}.

In order to develop a reproducible process, cell suspensions of *Pseudomonas* sp. Ac were repeatedly used in four additional operation cycles. Table 1 shows that the yields were generally > 95%, and that the cells did not lose any activity during each cycle of reaction. Rather, a slight tendency for an increase of the catalytic efficiency was observed with each cycle.

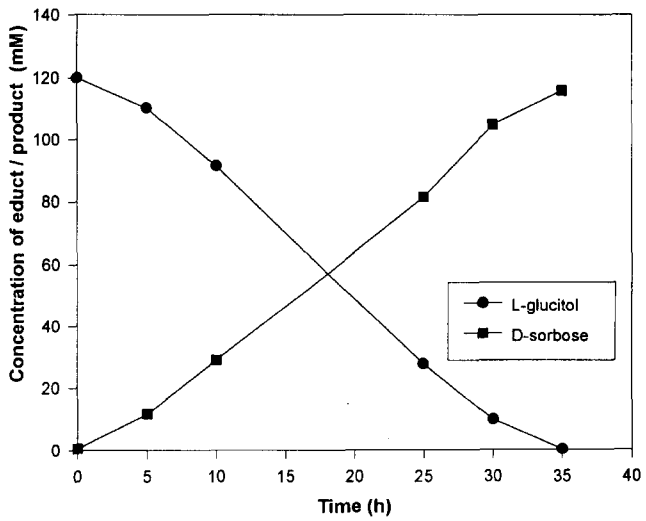

Fig. 1. Bioconversion of L-glucitol (**2**) into D-sorbose (**3**) by resting cells of *Pseudomonas* sp. Ac. Conditions are described in Experimental.

1. Experimental

Organism and growth conditions.—Precultures of *Pseudomonas* sp. Ac were grown with shaking (150 rpm) at 30 °C for 24 h in a minimal medium containing the following components in a final volume of deionized water (1 L): D-glucose (4.5 g); KH_2PO_4 (1.0 g); NH_4Cl (1.6 g); $MgSO_4 \cdot 7H_2O$ (0.4 g); NaCl (0.4 g); $CaCl_2 \cdot 2H_2O$ (0.05 g); 10 × trace element solution SL 4 (1 mL) [14]; 10 × vitamin solution (1 mL) [15]. The pH was adjusted to 6.0 with aq NaOH. When cell suspensions for bioconversions were required, the bacterium was grown in a 2-L bioreactor filled with 1.5 L of medium and inoculated with 150 mL of a preculture. In the bioreactor, cells were grown for 48 h at 30 °C with agitation (400 rpm), aeration (3 L/min), and pH regulation at 6.0. When the cell absorption (A_{436}) reached 11.4 the culture was harvested by centrifugation (26,000 *g*; 4 °C, 15 min) and washed twice with 50 mM potassium phosphate, pH 7.0.

Bioconversion.—The washed cells were suspended in 20 mM potassium phosphate, pH 6.6, and adjusted to a concentration of 15 mg (dry weight) per mL. The suspension (200 mL total volume) was shaken in the presence of substrate in a double-baffled Erlenmeyer flask at 150 rpm at 30 °C. Samples were taken at the time intervals indicated, cells were removed by centrifugation, and each supernatant solution was assayed for substrate and product. For repeated use of the cells in bioconversions they were washed and resuspended as above.

Starting material and product identification, and sugar purification.—Starting material and product in bioconversions were determined by HPLC in comparison with authentic L-glucitol [10] and D-sorbose (Sigma). Using a Benson-100 carbohydrate column (Benson Polymeric Inc., Reno, USA) [11,12] and water as the mobile phase at a flow rate of 0.85 mL min^{-1}, the retention times determined for L-glucitol and D-sorbose were 15.5 and 8.5 min, respectively. It should be noted that D-sorbose, the product of C-5 oxidation of L-glucitol, could be clearly distinguished from the hypothetical C-2 oxidation product L-fructose which had a retention time of 9.5 min. When the bioconversion was complete, cells were removed by centrifugation as above, and the resulting supernatant solution was centrifuged at 53,000 *g* for 30 min at 4 °C. Then the supernatant solution was concentrated in a Büchi rotary evaporator at 70 °C to volumes ranging from 20 to 40 mL. D-Sorbose was purified from this solution by ligand exchange chromatography on a Ca^{2+}-loaded Dowex 50W-X8 column [11,12]. D-

Table 1
Operational stability of cell suspensions of *Pseudomonas* sp. Ac in bioconversions. Conditions are described in Experimental

Operation cycle	Concentration of L-glucitol (mM)	Reaction time (h)	Yield (%)
1	50	26	94.3
2	56	18	95.1
3	60	21	97.8
4	61	23	96.7

Sorbose-containing fractions were pooled and concentrated by evaporation to dryness. The optical rotation was determined with a Perkin–Elmer 241 spectral polarimeter, using water as solvent.

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