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Note

Enzymatic synthesis of L-tagatose from galactitol with galactitol dehydrogenase from *Rhodobacter sphaeroides* D

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Abstract

The rare sugar L-tagatose was prepared with an overall yield of 78% by enzymatic C-5 oxidation of galactitol with co-substrate regeneration, and purification by ligand exchange chromatography. The sugar was identified by HPLC, melting point determination, optical rotation, and NMR spectrometry. © 1998 Elsevier Science Ltd.

Keywords: L-Tagatose; Galactitol; Galactitol dehydrogenase; Bioconversion

1. Introduction

Unnatural monosaccharides are potentially useful as nonmetabolizable sweeteners and as building blocks for the synthesis of interesting natural and biologically active products [1]. For many decades, L-sorbose is used as the starting material for the industrial production of L-ascorbic acid [2], and only recently has it been used as a precursor for the facile synthesis of the potent glycosidase inhibitor 1-deoxygalactonojirimycin [3]. Similarly, but more efficiently, L-tagatose (**2** Scheme 1) can be also used as starting material for the synthesis of 1-deoxygalactonojirimycin [3]. While L-sorbose is abundantly available, the accessibility of **2** is hampered by insufficient methods of production. **2** has been prepared chemically from 1,5-anhydro-D-galactitol, D-galactose and L-tartaric acid with yields of 3% [4], 38% [5,6], and 15% [7,8], respectively. More efficient are enzymatic routes, e.g., by use of L-fuculose 1-phosphate aldolase for the synthesis of L-tagatose 1-phosphate (86%) from dihydroxyacetone phosphate and L-glyceraldehyde [9] which can be dephosphorylated to **2** (79%) with phosphatase [10]. The isomerization of the rare sugar L-talose to **2** (75%) with L-rhamnose isomerase [9], the epimerization of L-sorbose into **2** (20%) with D-tagatose 3-epimerase [11], and the microbial conversion of galactitol to **2** (70%) with *Klebsiella pneumoniae* strain 40b [12] have also been demonstrated.

In this communication, we report an efficient enzymatic synthesis of **2** by regioselective oxidation at C-5 of the readily available polyol galactitol **1** Scheme 1 with partially purified galactitol dehydrogenase (GDH) [13]. Since oxidation of **1** to **2** by GDH is accomplished in the presence of catalytic amounts of NAD (NAD:galactitol = 1:28), an efficient system for continuous in situ regeneration of NAD with

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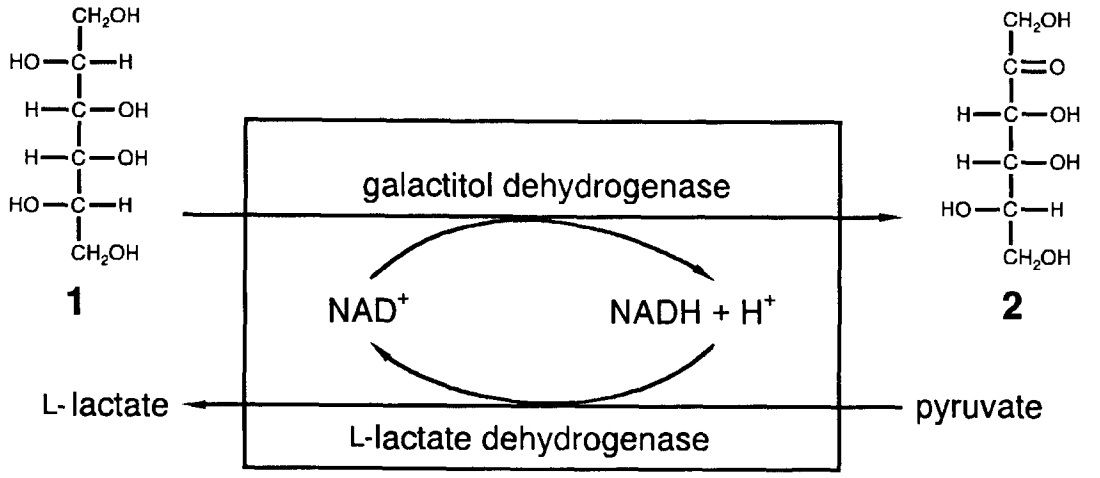

Scheme 1.

lactate dehydrogenase (LDH) is required [14] (Scheme 1). In this system, substrate oxidation is favoured by an alkaline pH, and by the low co-substrate concentration used in combination with an efficient re-oxidation of NADH with excess LDH, which altogether minimizes product inhibition of GDH by NADH.

Fig. 1 shows the time course of the bioconversion with partially purified GDH in the presence of 100 mM **1** (1.8 g in 100 mL) to give **2** in yields of 98%. This result was confirmed by two other experiments performed under the same conditions. However, at lower GDH concentrations than 130 U/100 mL (see Section 2) the bioconversions were incomplete and resulted in significant lower yields of **2** with prolonged reaction times. **2** was purified from the reaction solution of the bioconversion illustrated in Fig. 1 by ligand exchange chromatography [15,16,18] and recovered as solid material following lyophilization. A total of 1.4 g solid **2** was obtained corresponding to an overall yield of 78%. **2** was identified by ^{1}H and ^{13}C NMR spectrometry in comparison with authentic D-tagatose [17], HPLC comparison with authentic D-tagatose (retention time: 11.8 min), mp {129–130 °C (lit. [4]: 134–135 °C)}, and optical rotation {$[\alpha]_D^{20}$ +5.7 (c 1.0, water); lit. [11]: $[\alpha]_D^{25}$ +6.7 (c 8.4, water)}.

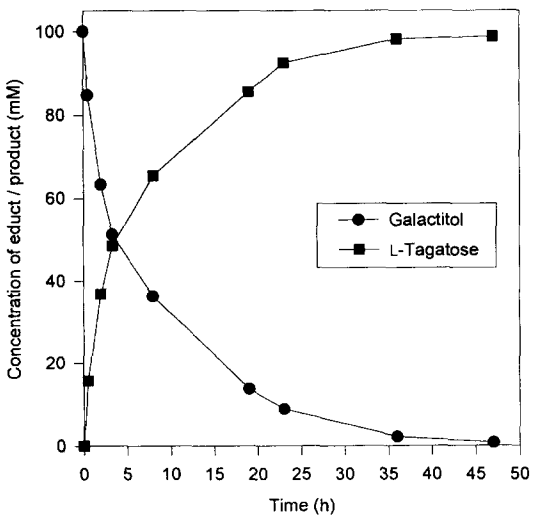

Fig. 1. Bioconversion of galactitol **1** to L-tagatose **2** by galactitol dehydrogenase from *Rhodobacter shaeroides* D. The operating conditions are described in Section 2.

2. Experimental

Organism and enzyme.—For the production of galactitol dehydrogenase (GDH), the phototrophic bacterium *Rhodobacter sphaeroides* D was used which is a galactitol utilizable gain of function mutant of the wild-type *R. sphaeroides* Si4 (DSM 8371) [13]. The organism was grown in a 2-L bioreactor on mineral medium with 6.0 g/L DL-malate as carbon source [13]. Galactitol dehydrogenase (GDH) was partially purified from cell extracts by ammonium sulfate precipitation and chromatography on Phenyl-Sepharose, and Q-Sepharose [13].

Bioconversion.—Bioconversions were performed in 100 mM Tris–HCl (pH 8.5) containing the following components in a total volume of 100 mL: galactitol, 100 mM; sodium pyruvate, 200 mM; magnesium chloride, 1 mM; NAD, 3.6 mM; GDH, 132 U; lactate dehydrogenase, 367 U. The solution was shaken in an Erlenmeyer flask at 150 rpm at 30 °C. Samples were taken at the time intervals indicated, and the supernatant was assayed for substrate and product.

Starting material and product identification, and sugar purification.—Educt and product in bioconversions were determined by HPLC in comparison with authentic galactitol and D-tagatose. A Benson-100 carbohydrate column (Benson Polymeric, Reno, USA) [14,15] was used, linked with a refractive index detector. The mobile phase was water at a flow rate of 0.85 mL min^{-1}. The retention times determined for galactitol and L-tagatose were 14.5 min and 11.8 min, respectively. When the bioconversion was complete, protein was precipitated and removed by centrifugation at 5000 g for 15 min at 4 °C. Then the supernatant solution was concentrated in a Büchi rotary evaporator at 40 °C to a volume of about 30 mL. L-Tagatose was purified from this solution by ligand exchange chromatography on a Ca^{2+} loaded Dowex 50W X8 column [15,16,18]. Fractions containing L-tagatose were pooled and concentrated by evaporation to dryness. L-Tagatose was identified by ^{1}H and ^{13}C NMR spectrometry in comparison with authentic D-tagatose prepared chemo-enzymatically from D-galactose [17]. The NMR spectra of L-tagatose were in complete agreement with those of authentic D-tagatose [17]. The optical rotation of L-tagatose was determined with a Perkin Elmer 241 spectral polarimeter following mutarotation for 16 h at room temperature: $[\alpha]_D^{20}$ +5.7 (c 1.0, water); {lit. [11] $[\alpha]_D^{25}$ +6.7 (c 8.4, water)}. The melting point of L-tagatose was 129–130 °C (lit. [4]: 134–135 °C).

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References

[1] R. Aljarin, E. Garcia-Junceda, and C.-H. Wong, *J. Org. Chem.*, **60** (1995) 4294–4295.
[2] T. Reichstein and A. Grussner, *Helv. Chim. Acta*, **17** (1934) 311–328.
[3] R. H. Furneaux, P.C. Tyler, and L.A. Whitehouse, *Tetrahedron Lett.*, **34** (1993) 3609–3612.
[4] C. Glatthaar and T. Reichstein, *Helv. Chim. Acta*, **20** (1937) 1537–1541.
[5] P.L. Barili, G. Berti, F. D'Andrea, and A. Gaudiosi, *Carbohydr. Res.*, **212** (1991) c5–c7.
[6] P.L. Barili, G. Berti, G. Catelani, F. D'Andrea, and A. Gaudiosi, *Gazz. Chim. Ital.*, **124** (1994) 57–63.
[7] T. Mukaiyama, K. Suzuki, and T. Yamada, *Chem. Lett.*, (1982) 929–932.
[8] T. Mukaiyama, Y. Yuki, and K. Suzuki, *Chem. Lett.*, (1982) 1169–1170.
[9] W.D. Fessner, J. Badia, O. Eyrisch, A. Schneider, and G. Sinerius, *Tetrahedron Lett.*, **33** (1992) 5231–5234.
[10] W.D. Fessner, Ger. Pat. 4111971 (1992), *Chem. Abstr.*, **117** (1992) 110129.
[11] H. Itoh and K. Izumori, *J. Ferment. Bioeng.*, 81 (1996) 351–353.
[12] T. Shimonishi, Y. Okumura, and K. Izumori, *J. Ferment. Bioeng.*, **79** (1995) 620–622.
[13] K.H. Schneider, G. Jäkel, R. Hoffmann, and F. Giffhorn, *Microbiology*, **141** (1995) 1865–1873.
[14] L.G. Lee and G.M. Whitesides, *J. Am. Chem. Soc.*, **107** (1985) 6999–7008.
[15] D. Schwartz, M. Stein, K.-H. Schneider, and F. Giffhorn, *J. Biotechnol.*, **33** (1994) 95–101.
[16] A. Huwig, H.-J. Danneel, and F. Giffhorn, *J. Biotechnol.*, **32** (1994) 309–315.
[17] S. Freimund, A. Huwig, F. Giffhorn, and S. Köpper, *J. Carbohydr. Chem.*, **15** (1996) 115–120.
[18] A. Huwig, S. Emmel, and F. Giffhorn, *Carbohydr. Res.*, **281** (1996) 183–186.