

An Efficient Synthesis of Sedoheptulose Catalyzed by Spinach Transketolase

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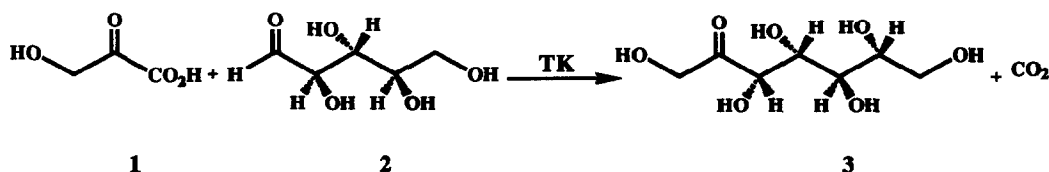
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Abstract : A practical procedure for transketolase catalyzed condensation of hydroxypyruvic acid with D-ribose has been used for the synthesis of D-sedoheptulose.

Sedoheptulose (D-*altro*-heptulose), discovered in 1917 by La Forge and Hudson in *Sedum spectabile*, is important in primary and secondary plant metabolism, particularly in the pentose phosphate pathways, the synthesis of sugars during photosynthesis and the biosynthesis of shikimic acid and aromatic aminoacids. D-sedoheptulose has been synthesized by biological and chemical methods. It is extracted from plants such as *Sedum spectabile*^{1,2} in which it is abundant, and from the fruit of *Coriaria japonica*³. It is also obtained by bioconversion from calcium gluconate using *Flavobacterium TSC A*⁴. Two chemical syntheses of sedoheptulose derivatives have been described: the first being the synthesis of sedoheptulose hexaacetate in four steps from cadmium D-altronate⁵, the second that of sedoheptulose-7-phosphate from 2-nitroethanol and D-ribose-5-P⁶. These chemical methods require many steps and giving racemates with yields that are poor (of the order of 15 %), though better than those obtained by the biological route.

We describe here a one-step enzymatic synthesis of D-sedoheptulose 3, catalyzed by spinach transketolase. This enzyme is involved *in vivo* in the building of sugars by carbon-carbon bond formation, and in particular by an aldolisation type reaction, although not strictly an aldolase. It catalyses the reversible transfer of a hydroxyacetyl group from a ketose donor to an aldose acceptor in the presence of thiamine pyrophosphate and magnesium. This enzyme is a useful catalyst for the synthesis of ketoses when hydroxypyruvate 1 is used as the donor substrate, because then the condensation becomes irreversible (Scheme 1).



Scheme 1
Enzymatic synthesis of D-sedoheptulose

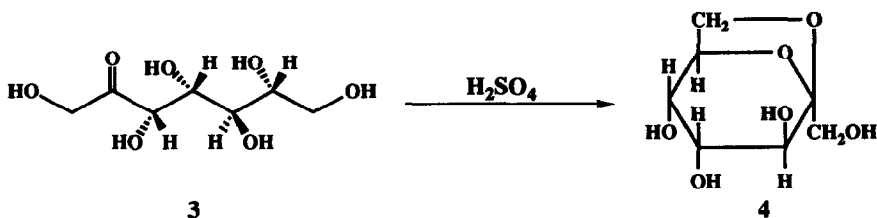
We showed previously⁷ that spinach transketolase will accept numerous aldehydes as substrates including D-ribose 2. This aldehyde is a good substrate for transketolase; the ratio of initial reaction rates for ribose and glycolaldehyde, the best known substrate for this enzyme, is 0.30. We have shown that syntheses can be carried out with lower rates, in particular of 4-deoxy-L-erythrulose⁷ where $V_{\text{acetaldehyde}}/V_{\text{glycolaldehyde}}$ is 0.12.

The use of baker's yeast transketolase in the synthesis of monosaccharides has been developed in the last two years and syntheses using this enzyme have been reported recently⁸⁻¹⁰.

For the synthesis of D-sedoheptulose we condensed hydroxypyruvate (7 mmoles) onto D-ribose (7 mmoles) in the presence of spinach transketolase (150 U) and its cofactors¹¹.

Spinach transketolase is stereospecific towards α -hydroxylated aldehydes such as D-ribose; the reaction give only D-sedoheptulose, with a yield of 70 % (about 1 g, $[\alpha]_D^{25} = + 8$). Heptulose was separated from the reaction mixture by filtration through H^+ resin.

As the identification of this heptulose in its free form is relatively complex, we prepared its derivative, 2,7-anhydro-D-sedoheptulose 4 by the action of hot concentrated sulphuric acid¹².



Scheme 2
Synthesis of 2,7-anhydro-D-sedoheptulose

Crystalline 2,7-anhydro-D-sedoheptulose¹³ was obtained with a yield of 60 %. Its physical constants agree with those reported in the literature² ($[\alpha]_D^{25} = - 149$, m.p. = 156°C). Its structure was confirmed by ^{13}C NMR¹³.

It is of note that fructose-1,6 diphosphate aldolase, an enzyme often used for the synthesis of monosaccharides^{14,15}, cannot afford sedoheptulose since erythrose, the aldose necessary for the reaction is not a substrate for this enzyme¹⁶. In addition, the product obtained directly from the transketolase catalyzed experiment is not phosphorylated, greatly simplifying the procedure for monitoring the progress of the reaction as well as the isolation of the product.

Thus the transketolization reaction is useful for the efficient and easy synthesis of D-sedoheptulose : this compound is obtained in a single step from the enzyme and readily accessible materials with a very good yield.

References and notes

- 1 - F. B. La Forge, C. S. Hudson, *J. Biol. Chem.*, **1917**, *30*, 61.
- 2 - N. K. Richtmyer, *Methods in Carbohydrate Chemistry*, **1962**, *1*, 167.
- 3 - T. Okuda, K. Konishi, *Yakugaku Zasshi*, **1968**, *88*, 1329.
- 4 - M. Takeshita, M. Nakamura, Y. Ashikari, A. Yasojima, *Jap. Patent 21760(66)*, **1964**.
- 5 - M. L. Wolfrom, J.M. Berkebile, A. Thompson, *J. Am. Chem. Soc.*, **1952**, *74*, 2197.
- 6 - B. A. Mc Fadden, L.L. Barden, N.W. Rokke, M. Uyeda, T.J. Siek, *J. Am. Chem. Soc.*, **1967**, *87*, 5505.
- 7 - C. Demuynck, J. Bolte, L. Hecquet, V. Dalmas, *Tetrahedron Lett.*, **1991**, *32*, 5085.
- 8 - D. C. Myles, P. J. Andrulis, G. M. Whitesides, *Tetrahedron Lett.*, **1991**, *32*, 4835.
- 9 - F. Effenberger, V. Null, T. Ziegler, *Tetrahedron Lett.*, **1992**, *33*, 5157.
- 10 - Y. Kobori, D.C. Myles, G.M. Whitesides, *J. Org. Chem.* , **1992**, *57*, 5899.
- 11 - Typical experiments :
30 mL of glycyl-glycine buffer 0.05 M pH 7.5, containing hydroxypyruvate (7 mmol), D-ribose (7 mmol), thiamine pyrophosphate (0.04 mmol), MgCl₂ (0.1 mmol) and 150 U of transketolase was deoxygenated with nitrogen and left at 25°C in the dark. After complete disappearance of hydroxypyruvate (3.5 h), ethanol (105 mL) was added and the precipitate discarded. The solution was concentrated under reduced pressure and the sugar isolated and purified by chromatography on cation exchanger (H⁺). The fractions that contained the sugar were pooled, adjusted to pH 6 with anion exchanger (Amberlite) and concentrated.
- 12 - Typical experiments :
Concentrated sulphuric acid (0.5 mL) was added to sedoheptulose (300 mg), and the mixture was heated 5 h. on a steam bath to convert sedoheptulose into sedoheptulosan. The solution was cooled, neutralised with solid barium carbonate, filtered, deionized by passage through a pair of ion-exchange resins, such as Dowex 50W-X2 (H⁺) and Amberlite IRA 93 (OH⁻), and concentrated under reduced pressure to a thick syrup. Dissolution of the syrup in hot methanol, followed by cooling and inoculation with a seed crystal yielded anhydrous sedoheptulosan. The product could be recrystallized from methanol and separated as large white crystals.

- 13 - 2,7-anhydro-D-sedoheptulose : m.p. = 156°C, $[\alpha]_D^{25} = -149$ (c = 0.3, H₂O), ¹³C NMR (300 MHz), CDCl₃, δ : 63.0 (C7); 69.6 (C1); 73.2 (C4 and C5); 75.4 (C6); 80.9 (C3); 110.7 (C2).
- 14 - T. Kajimoto, K.K. Liu, R.L. Pederson, Z. Zhong, Y. Ichikawa, J.A. Porco, C.H. Wong, *J. Am. Chem. Soc.*, **1991**, *113*, 6187.
- 15 - R.R. Hung, J.A. Straub, G.M. Whitesides, *J. Org. Chem.*, **1991** *56*, 3849.
- 16 - M.D. Bednarski, E.S. Simon, N. Bischofberger, W.D. Fessner, M.J. Kim, W. Lees, T. Saito, H. Waldmann, G.M. Whitesides, *J. Am. Chem. Soc.*, **1989**, *111*, 627.