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ASYMMETRIC REDUCTION OF KETONES BY GLYCEROL DEHYDROGENASE FROM *GEOTRICUM*

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Abstract; Glycerol dehydrogenase from *Geotricum* was used as a catalyst for asymmetric reduction of ketones. A 2-propanol-NAD^+ couple was employed to supply NADH.

Asymmetric reduction by biological systems has become important in organic synthesis.[2] The use of a dehydrogenase as a reducing catalyst for the reduction is a convenient method because of high stereospecificity.[3] However, the number of dehydrogenases familiar in laboratories of organic chemistry is limited, and a search for a new dehydrogenase easy to be handled is long awaited. In this paper, we would like to report stereochemistry of the reduction of ketones by glycerol dehydrogenase (EC 1.1.1.6) from *Geotricum candidum* (GGDH).[4]

Ketones were reduced by GGDH with a 2-propanol-NAD^+ couple to supply NADH continuously and absolute configurations of the products were determined. Thus 1 mmol of a substrate was reduced by GGDH (9.3 unit), 0.012 mmol of NAD^+, and 10 mmol of 2-propanol in 10 ml of pottasium phosphate buffer (0.1 M, pH 7.0) for 1-2 days. Then the organic materials were extracted with ethyl acetate and subjected to a preparative GC yielding the reduced product. Chemical yields of the products were between 30 to 60 % for smoothly reacting substrates. The absolute configuration of the product was determined by comparing the sign of optical rotation with that of the authentic compound or by comparing the retention time on GC or HPLC of the MTPA derivative with that from the authentic compound. The enantiomer excess (e.e.) was determined by GC, HPLC or NMR spectrum of the MTPA derivative. Results are listed in Scheme 1.

GGDH reduced α-keto esters smoothly. The substrate-specificity is rather wide. Ethyl esters of pyruvic to 2-oxohexanoic acids were thus reduced to give the corresponding (*R*)-hydroxy esters. Ethyl 2-oxoheptanoate was reduced only slowly. Methyl 3-methyl-2-oxobutanoate was reduced to methyl (*R*)-3-methyl-2-hydroxybutanoate smoothly but its analog, ethyl 4-methyl-2-oxopentanoate, was not reduced. In a series of β-keto easters, ethyl 4-chloro-3-oxobutanoate was reduced to ethyl (*S*)-4-chloro-3-hydroxybutanoate while ethyl 3-oxobutanoate was reduced only slowly. All α- and β-keto esters so far studied with the present reduction system gave D-hydroxy esters stereospecifi-

cally. Reduction of 1-chloro-2-propanone took place smoothly but the enantioselectivity of this reduction was not satisfactory (e.e.= 67 %). Thus GGDH recognizes the ester function very efficiently but not a chloride substituent, which is polar as well.

The substrate-specificity of GGDH is different from glycerol dehydrogenases (GDH) from other microbes.[5,6] GDH from *Enterobacter* and *Celluromonous*[5] does not reduce α- and β-keto esters. GDH from *Celluromonous* reduces 1-chloro-2-propanone but only with a very slow rate (1/4000 compared with GGDH). GDH from *Mucor*[6] reduces 2-alkanone while GGDH does not.

The advantage of GGDH for asymmetric synthesis is that we can use 2-propanol as a reducing reagent for turning the NAD^+-NADH cycle. This advantage is useful for organic synthesis, especially for practical uses, because another dehydrogenase is not required for reproducing the coenzyme and 2-propanol and acetone, which is the oxidized product during the reduction of a substrate, can be removed easily from the reaction mixture. We believe that GGDH is a useful dehydrogenase for organic synthesis.

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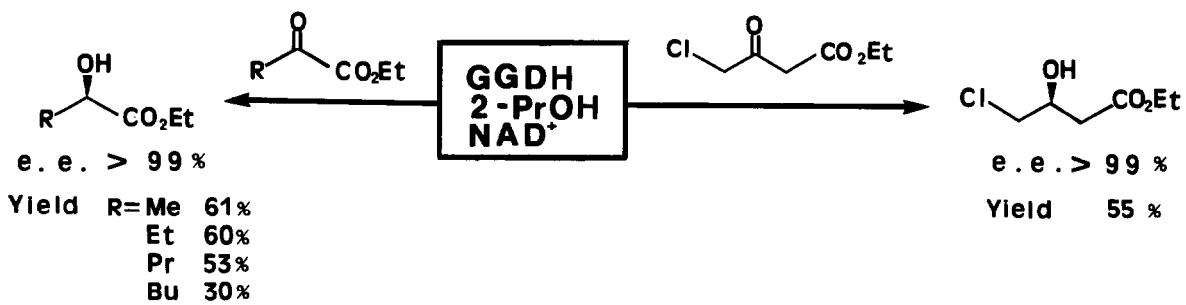
Scheme 1. Asymmetric reduction of ketones by GGDH.



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