Bioorganic & Medicinal Chemistry Letters, Vol.2, No.6, pp. 619-622, 1992
Printed in Great Britain

0960-894X/92 $5.00 + .00

ASYMMETRIC REDUCTION OF KETOESTERS WITH ALCOHOL DEHYDROGENASE FROM *THERMOANAEROBACTER ETHANOLICUS*

Changsheng Zheng, Van T. Pham and Robert S. Phillips*
Department of Chemistry, University of Georgia
Athens, GA 30602, U. S. A.

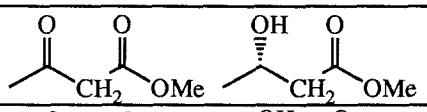
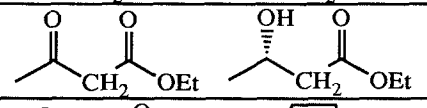
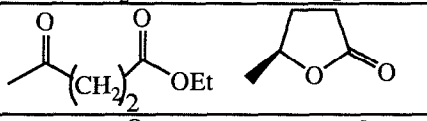
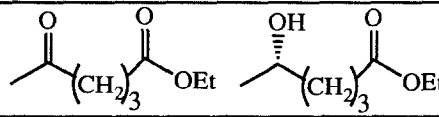
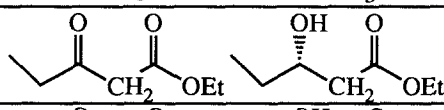
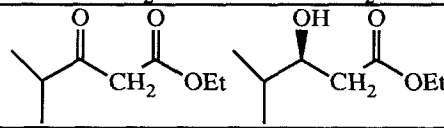
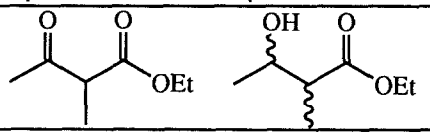
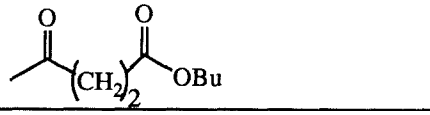
(*Received* 20 *March* 1992)

Abstract: The secondary alcohol dehydrogenase (SADH) of *T. ethanolicus* was produced by controlling the growth temperature at 50 °C. Chiral hydroxyesters were obtained by the asymmetric reduction of ketoesters catalyzed by SADH. Reduction of ethyl levulinate gave (S)-γ-butyrolactone in 84% yield and greater than 99% e.e..

It is well known that baker's yeast can be used for the preparation of hydroxyesters through the reduction of ketoesters.[1] However, the stereoselectivity may be low and unreproducible, because of the competitive participation of several dehydrogenases with different enantioselectivity [S-enzyme(s) and R-enzyme(s), respectively].[2] Some modifications can be made to obtain high stereoselectivity; for example, the introduction of a third reagent into the reaction systems changes the stereoselectivity of the reaction,[3] and a semibatch procedure with carefully controlled addition of the substrate and sucrose can obtain comparatively high e.e.(95-97%).[4] Report of the reduction of ketoesters by other alcohol dehydrogenases are rare, but a recent report showed that an alcohol dehydrogenase from *Thermoanaerobium brockii* can reduce a range of ketoesters from methyl 5-oxohexanoate to methyl 8-oxononanoate with high e.e.%.[5] There is still a need for new enzymes or microorganisms which have improved selectivity and expand the scope for the asymmetric reduction of ketoesters. Here we describe the results of our studies of the asymmetric reduction of ketoesters with the secondary alcohol dehydrogenase from the thermophilic anaerobic bacterium, *Thermoanaerobacter ethanolicus* (ATCC 31550).

T. *ethanolicus* grown on D-glucose contains both primary and secondary alcohol dehydrogenases. The secondary alcohol dehydrogenase (SADH) is formed early during the growth cycle, whereas the primary alcohol dehydrogenase (PADH) is formed late in the growth cycle.[6] To prepare the enzyme for synthesis, cells were obtained at the early growth phase by controlling the growth temperature (50 °C) and time (22 hrs). Under these conditions, more than 99% of the alcohol dehydrogenase activity was obtained as SADH, whereas growing cells at 60 °C for 22 hrs produces PADH only (the percentage content of PADH and SADH in cell extracts was determined by comparison of the initial velocities of production of NADPH by enzymatic oxidation of 1-butanol

Table 1 Asymmetric reduction of ketoesters with SADH[a]

Entry	substrate	product	observed % yield	$[\alpha]_D$ (°)	e.e.[b] %	absolute config.
1			53	+ 36.6 (c=1.80, $CHCl_3$)	95	S[7a]
2			56	+ 40.4 (c=1.44, $CHCl_3$)	96	S[7b]
3			84	- 29.5 (c=1.30, $CHCl_3$)	99	S[7c]
4			45	+ 11.5 (c=2.41, $CHCl_3$)	98	S[5]
5			6	+ 32.1 (c=2.10, $CHCl_3$)	98	S[7b]
6			35	- 32.5 (c=1.80, $CHCl_3$)	98	S[7b]
7			7			
8			no reaction			

a. Reaction solutions containing the following: ketoester (0.5%, v/v), 2-propanol (15%, v/v), SADH (2.0 units/ml), NADP (0.05 mM), mercaptoethanol (3 mM), and tris-HCl buffer (50 mM, pH 8), were stored at 50 °C. The reduction was followed by GC until no more products formed and worked up.

b. 15-20 mg hydroxyesters were mixed with 3 molar equivalents of MTPA, 2 mg DMAP and 50 mg 1,3-diisopropylcarbodiimide in 5 ml dry CH_2Cl_2 and stirred at room temperature for 2 hrs. The resulting diastereomeric MTPA esters were analyzed by GC-MS [25m cross-linked methyl silicone capillary, HP-1, 120 °C (2 mins) / 5 °C per min / 250 °C] or GC [25m capillary, DB-wax, 150 °C (2mins) / 3 °C per min / 240 °C].

and 2-propanol, while cell extracts were prepared by sonication of cell suspensions in 0.02 M Tris buffer, pH 7.6.[6]).

Table 1 shows the results of reduction of ketoesters. The observed yields were determined by GC analysis. Pure compounds were obtained by flash chromatography (silica gel, proper proportion of hexane and ethyl acetate). The absolute configurations were assigned by comparison of the sign of rotation with literature data,[5,7] while the stereochemical purity of hydroxyesters was determined by the analysis of the corresponding methoxy(trifluoromethyl)phenyl acetate (MTPA esters). We used direct esterification of MTPA acid, coupling with hydroxyesters catalyzed by 1,3-diisopropylcarbodiimide and 4-dimethylaminopyridine (DMAP). In the study of model compounds, we found that the ratio of the corresponding diastereoisomeric MTPA esters did not correspond to the actual proportion of enantiomers in the studied mixture. This behavior has also been found for the direct esterification of MTPA acid catalyzed by 2-chloro-1-methylpyridium iodide and DMAP.[8] The above discrepancy is due to incomplete reaction of the alcohols, and the reaction mixture was kinetically enriched in the faster arising diastereoisomer. We found that preferential formation of one of the diastereoisomeric esters was avoided by using three equivalents of MTPA.

Enzymatic reduction of methyl acetoacetate (**1**) and ethyl acetoacetate (**2**) yielded (S)-3-hydroxybutanoate esters in 54% and 56% yield with great than 95% e.e.. In the case of ethyl levulinate (**3**), the product 4-hydroxyester was not isolated, due to its facile cyclization to give γ-valerolactone. We observed that as the reaction time increased, the peak area of the hydroxyester in GC decreased to zero, whereas the peak of lactone increased. The yield of (S)-γ-valerolactone (84%) was significantly higher than the hydroxyesters, probably due to cyclization driving the reduction equilibrium toward the lactone product. The stereochemical purity of the γ-valerolactone was determinated with the europium shift reagent [3-(trifluoromethylhydroxymethylene)-(+)-camphorato)]-europium(III) ($Eu(tfc)_3$); comparison of the NMR spectra of the racemic and chiral compounds showed that the optical purity of the latter exceeds 99%. We also noted that the initial concentration of ethyl levulinate affects the yield significantly. The yield using 0.5% substrate (84%) is higher than 1% substrate (50%), although there is no difference for other substrates. Ethyl 5-oxohexanoate (**4**) gave an S-(+) hydroxyester with lower yield than ethyl acetoacetate (**2**) and did not produce the cyclization product, (S)-(-)-6-methyl valerolactone, under reaction conditions. This difference is likely due to the cyclization of 5-membered rings being kinetically more favorable than for 6-membered rings. Ethyl propionylacetate (**5**) showed a much lower yield. It is interesting that ethyl isobutyrylacetate (**6**), with an isopropyl ketone group, produced higher yield than ethyl propionylacetate (**5**), with an ethyl ketone group. Remarkably, the product of reduction of ethyl isobutyrylacetate (**6**) exhibits the opposite sign of rotation and the opposite absolute configuration (although it is still assigned the S configuration) than

the product of reduction of ethyl propionylacetate (**5**). When ethyl levulinate (**3**) was replaced by butyl levulinate (**8**), no reaction was observed. This result can be explained by the pocket model of the active site of SADH proposed in our previous paper;[9] very long chain molecules do not fit into the active site. Methyl-2-methylacetoacetate (**7**) gave a mixture of *threo* and *erytho* products with low yield and poor stereoselectivity, and we did not study the details of the stereochemistry further.

The SADH from *T. ethanolicus* can thus perform reduction of some ketoesters to give good yields of hydroxyesters (or lactones) with high stereochemical purity. The use of these products as chirons for stereospecific synthesis is now under investigation in our laboratory.

Acknowledgement We thank Nancy Bond and William Reid for supplying a culture of *T. ethanolicus* and for assistance in growth of the organism.

References

1 (a) Servi, S., *Synthesis*, **1990**, 1-25. (b) Csuk, R. and Brigitte, G., *Chem. Rev.*, **1991**, 91, 49-97.

2 (a) Hintzer, K.; Koppenhoefer, B. and Schurig, V., *J. Org. Chem.*, **1982**, 47, 3850-3854. (b) Keck, G. and Murry, J., *J. Org. Chem.*, **1991**, 56, 6606. (c) Sih, C. and Chen, C., *Angew. Chem. Int. Ed. Engl.*, **1984**, 23, 570-578. (d) Zhou, B.; Gopalan, A.; Vanmiddlesworth, F.; Shieh, W. and Sih, C., *J. Am. Chem. Soc.*, **1983**, 105, 5925-5926. (e) Heidlas, J.; Engel, K. and Tressl, R., *Eur. J. Biochem.*, **1988**, 172, 633-639.

3 (a) Nakamura, K.; Kawai, Y.; Nakjima, N. and Ohno, A., *J. Org. Chem.*, **1991**, 56, 4778-4783. (b) Ushio, K.; Ebara, K. and Yamashita, T., *Enzyme Micro. Technol.*, **1991**, 13, 834-838.

4 Wipt, B.; Kupfer, E.; Bertazzi, R. and Leuenberger, H., *Helv. Chim. Acta.*, **1983**, 66, 485-488.

5 Keinan, E.; Seth, K.; Lamed, R.; Ghirlando, R. and Singh, S., *Biocatalysis.*, **1990**, 3, 57-71.

6 Bryant, F.; Wiegel, J. and Ljundahl, L., *Appl. Environ. Microbiol.*, **1988**, 54, 460-465.

7 (a) *Dictionary of organic compounds;* Buckingham, J. ed.; Chapman and Hall: New york, 1982; Vol 3, p. 3011. (b) Buisson, D.; Azerad, R.; Sanner, C. and Larcheveque, M., *Tetrahetron: Asymmetry*, **1991**, 2, 987-988. (c) Mori, K., *Tetrahetron*, **1975**, 31, 3011.

8 Svatos, A.; Valterova, I.; Saman, D. and Vrkoc, J., *Collect. Czech. Chem. Common.*, **1990**, 55, 485-490.

9 Pham, V. and Phillips, R. S., *J. Am. Chem. Soc.*, **1990**, 112, 3629-3632.