Tetrahedron: Asymmetry Vol. 2, No. 10, pp. 987-988, 1991
Printed in Great Britain

0957-4166/91 $3.00+.00
Pergamon Press plc

Stereocontrolled Reduction of β-ketoesters by *Geotrichum candidum*. Preparation of D-3-hydroxyalkanoates

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(*Received* 30 *August* 1991)

Keywords: microbial reduction; Geotrichum candidum; β-ketoesters; D-3-hydroxyalkanoates.

Abstract: Geotrichum candidum has been used for the preparative reduction of β-oxoalkanoic esters to the corresponding D-β-hydroxyesters. The stereospecificity for ethyl 3-oxobutanoate or 3-oxopentanoate is markedly increased by preincubation of the mycelium before addition of the substrate.

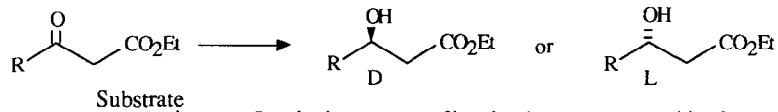
Baker's yeast has been frequently used for the preparation of β-hydroxyesters through the reduction of β-ketoesters[1,2]. However, only one specific configuration is obtained and the enantiomeric excess may be low, probably because several dehydrogenases with opposite stereospecificities are simultaneously active[2,3]. For example, the reduction of ethyl acetoacetate and ethyl 3-oxopentanoate by yeast affords respectively the corresponding L-3-hydroxybutanoate in 70-90% e.e. (depending on the concentration of the substrate)[4] and D-3-hydroxypentanoate in only 12-40% e.e[5,6]. Alternative methods for obtaining the opposite enantiomer of 3-hydroxybutanoate, such as the depolymerization of microbial poly (R)-hydroxybutyrate[7], or a chemical hydrogenation in the presence of chirally modified catalysts[8] have been developed with some success.

On the other hand, several methods have been described to control the stereochemistry of the microbial reduction: chemical modification of the substrate[5,9], or growth and incubation of the yeast in conditions where undesirable enzymes are inactivated[6,10-13]. Another method is the use of alternate microorganisms which may present opposite or better stereospecificity. Wipf *et al.*[4] have described a large scale reduction of ethyl acetoacetate into ethyl D-3-hydroxybutanoate by a strain of *Geotrichum candidum;* however, the resulting yield and enantiomeric excess were poorly reproducible. We have shown, in a previous paper, that a simple preincubation of the mycelium of *G.candidum* in water eliminated a dehydrogenase activity with a (3S) *syn*-specificity in the reduction of α-methyl acetoacetate esters[14]. Based on this strategy, we want to report here a method for the preparation of ethyl D-3-hydroxyalkanoates in good yields and high enantiomeric excesses.

G. candidum (a locally isolated strain which grows as a filamentous biomass and thus can be easily filtered) was grown for 2 days at 27°C on a nutrient liquid medium[14]. Reduction was performed in two different sets of conditions: A, the substrate was directly added to the grown culture; B, the mycelium was separated by filtration, washed and resuspended in water, then preincubated at 27°C during 24 hours before the substrate was added. No additional glucose was supplied during the incubation in both conditions. When the reduction has been completed, the mycelium was filtered, washed with water and the filtrate, saturated with sodium chloride, was extracted with ethyl acetate. After evaporation of the solvent, the hydroxyester was distilled under vacuum.

The results of the reduction of several homologous 3-oxoesters are shown in the Table. With the shorter 3-oxoalkanoates (R= Me, Et or i-Pr) the preincubation of the mycelium increased the selectivity of the reduction, and for example ethyl D(R)-3-hydroxypentanoate was easily obtained as an optically pure product in a (non-opti-

Table. Reduction of Ethyl 3-oxoalkanoates with *G.candidum*



R	Substrate concentration ($g.l^{-1}$)	Incubation conditions[a]	hours	% reduction observed (%yield)[b]	Absolute configuration	(e.e.%)[c]
CH_3	6	A	4	82	L(S)	7
	6	B	7	95	D(R)	52
			24	100	D(R)	60
			72	100	D(R)	96
	8	B	96	100 (75)	D(R)	99
C_2H_5	1	A	8	92	D(R)	80
	1	B	24	100	D(R)	99
	5	B	48	95 (52)	D(R)	99
n-C_3H_7	4	A	24	90 (60)	D(R)	98
	4	B	24	55	D(R)	99
i-C_3H_7	1	A	22	95	D(S)	27
	1	B	23	85	D(S)	85
	2.6	B	70	90 (40)	D(S)	94
n-C_5H_{11}	1	A	24	68 (21)	D(R)	90
	3	B	24	21	D(R)	82
n-C_7H_{15}	1	A	24	5[d]	D(R)	89

[a] See text. [b] % reduction was measured by GPC; % yield as isolated product after distillation. [c] determined by GPC (DBwax or BP-10 capillary columns) after derivatization with (S)-O-acetyllactyl chloride[15]. [d] a large amount of 2-nonanone was found

mized) 52% yield. Ethyl D-3-hydroxyhexanoate was directly obtained in 98% e.e. and good yield using fresh mycelium (Method A). However, the rate of the reduction and the yield of reduced esters decreased markedly for higher homologs, as the result of the competitive formation of (n-1) 2-ketones, probably through hydrolysis and decarboxylation of oxoesters. Method B is more satisfactory for the preparation of D(R)-3-hydroxybutanoate: as shown in the Table, a high optical purity is only achieved if incubation is continued after the end of the reduction. A detailed study of the mechanisms responsible for such an improvement will be soon reported[16].

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