ELSEVIER

Journal of Molecular Catalysis A: Chemical 107 (1996) 405–407

JOURNAL OF MOLECULAR CATALYSIS A: CHEMICAL

Microbial synthesis of (+) and (−) methyl 4-chloro-4,4-difluoro-3-hydroxybutanoate

Arrigo Forni *, Irene Moretti, Fabio Prati, Giovanni Torre

Università di Modena, Dipartimento di Chimica, via Campi 183, 41100 Modena, Italy

Abstract

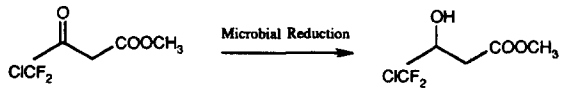
Microbial reduction with baker's yeast or *Aspergillus niger* of methyl 4-chloro-4,4-difluoro-3-oxobutanoate **1** afforded the optically active methyl (−)-4-chloro-4,4-difluoro-3-hydroxybutanoate **2**. When yeast reduction was carried out in the presence of allyl bromide the optically active methyl (+)-4-chloro-4,4-difluoro-3-hydroxybutanoate **2** was obtained.

Keywords: Asymmetric reduction; Microbial reduction; Stereochemical control; Chiral esters; Fluoro-β-hydroxyesters

1. Introduction

Optically active fluoro-organic compounds are of great interest, particularly as they can constitute chiral synthons for the preparation of bioactive molecules [1]. The microbiological approach to the transformation of fluorinated substrates, to obtain optically active derivatives, is of especial interest owing to the advantages offered by the biocatalytic processes [2]. In particular, the appropriate choice of the enzyme or of the micro-organism, as well as selective inhibition of the micro-organism itself, enables the stereochemistry of the reaction to be controlled and both the enantiomers to be obtained from a given substrate [2,3].

Here we report the results obtained in the microbial reduction with baker's yeast or *Aspergillus niger* of methyl 4-chloro-4,4-difluoro-3-oxobutanoate **1**.



1 (–) or (+) **-2**

2. Experimental

Fresh bakers' yeast (FALA, Strasbourg) and commercially available glucose were used for the reactions. Methyl 4-chloro-4,4-difluoro-3-oxobutanoate **1** was purchased from Fluorochem; additives were purchased from Aldrich except for allyl *p*-toluenesulphonate which was obtained from allyl alcohol and *p*-toluenesulphonyl-chloride in aqueous NaOH 20%. Optical rotations were measured on a Perkin-Elmer 241 polarimeter at 20°C. GLC analyses were performed on a Hewlett-Packard 5890 A gas chromatograph; the conversions were evaluated on a DB1 column (30 m × 0.53 mm I.D. and 5 μm film phase) from J and W Scientific, while the enantiomeric excesses were evaluated on a

* Corresponding author.

SSDI 1381-1169(95)00240-5

chiral G-DEX 120 column (30 m × 0.25 mm I.D. and 0.25 μm film phase) from Supelchem. ^{1}H-NMR spectra were recorded in $CDCl_3$ on a Bruker AMX 400 WB spectrometer; chemical shifts being reported in δ values from TMS as internal standard, coupling constants (J) are given in Hz. Elemental analyses were determined with a Carlo Erba Elemental Analyzer Mod. 1106. The yeast reductions were carried out as previously described [4].

2.1. Reductions with aspergillus niger (IPV283)

The micro-organism *Aspergillus niger* was grown in three different culture media [5]: Czapek, containing glucose (15 g), $NaNO_3$ (1.5 g), $MgSO_4$ (0.25 g), KCl (0.25 g), $FeSO_4$ (0.005 g) and K_2HPO_4 (0.5 g) in deionized water (1 l) adjusted to pH 7.3; MPG, containing glucose (20 g), peptone (5 g), malt (20 g) in deionized water (1 l) adjusted to pH 7.3; Sabouraud, containing glucose (40 g) and peptone (10 g) in deionized water (1 l) adjusted to pH 5.6. In a typical experiment, the culture was placed in 200-ml conical flask containing 50 ml of medium stirred at 120 rpm at 25–27°C. After 48 h the content of the flask was filtered and the mycelium washed repeatedly with 8% NaCl solution. The recovered wet mycelium was placed in 50 ml of the same fresh broth and the substrate (0.5 mmol) was then added. The suspension was stirred at 120 rpm at 25–27°C. After the required time (1 or 4 days) the reaction mixture was filtered and the filtrate extracted with diethyl ether. The combined extracts were dried on Na_2SO_4 and evaporated off. The crude residue was analyzed in GLC for conversion and enantiomeric excess.

2.2. Methyl(−)-4-chloro-4,4-difluoro-3-hydroxybutanoate 2

In 250 ml of distilled water containing yeast (28 g), glucose (15 g) and the additive allyl alcohol (3 g/l), the substrate **1** (10 mmol) was added [4]. The suspension was extracted with diethyl ether after incubation at 27–30°C for 24 h. The combined extracts, dried on Na_2SO_4 and evaporated off, showed 100% of conversion. The crude residue was chromatographed on silica gel (light petroleum/ethyl ether 7:3 as eluent) and, after distillation, the hydroxybutanoate **2** was recovered in 61% yield, with a 98°C b.p. at 20 mmHg, $[\alpha]_D$ −16.4 ($CHCl_3$), 71% ee. ^{1}H-NMR ($CDCl_3$), δ ppm: 2.68 (1H, dd, CH_2–CH, J = 16.6, 9.2 Hz), 2.78 (1H, dd, CH_2–CH, J = 16.6, 3.0 Hz), 3.62 (1H, d, OH, J = 5.6 Hz), 3.74 (3H, s, OCH_3), 4.47 (1H, m, CH–OH, J = 3.1, 5.6, 7.8, 9.2 Hz). Elem. anal., found% (calculated for $C_5H_7F_2ClO_3$): H 3.98 (3.74), C 31.54 (31.85).

2.3. Methyl (+)-4-chloro-4,4-difluoro-3-hydroxybutanoate 2

In 125 ml of distilled water containing yeast (14 g), glucose (7.5 g) and the additive allyl bromide (3 g/l), the substrate **1** (5 mmol) was added [4]. After the same treatment as described above the crude residue showed 41% of conversion. It was chromatographed on silica gel (light petroleum/ethyl ether 7:3 as eluent) and treated with semicarbazide in *n*-hexane to eliminate the unchanged ketone. After distillation, we obtained the hydroxybutanoate **2** in 30% yield with a 97–98°C b.p. at 20 mmHg, $[\alpha]_D$ + 15.9 ($CHCl_3$), 68% ee. ^{1}H-NMR ($CDCl_3$), δ ppm: 2.68 (1H, dd, CH_2–CH, J = 16.6, 9.2 Hz), 2.78 (1H, dd, CH_2–CH, J = 16.6, 3.0 Hz), 3.62 (1H, d, OH, J = 5.6 Hz), 3.74 (3H, s, OCH_3), 4.47 (1H, m, CH–OH, J = 3.0, 5.6, 7.8, 9.2 Hz). Elem. anal., found% (calculated for $C_5H_7F_2ClO_3$): H 4.01 (3.74), C 31.54 (31.85).

3. Results and discussion

The yeast reduction of methyl 4-chloro-4,4-difluoro-3-oxobutanoate **1** afforded the methyl (−)-4-chloro-4,4-difluoro-3-hydroxybutanoate **2** with 100% conversion and in 20–65% enantiomeric excesses depending on the ratio substrate/yeast/glucose (Table 1). In order to improve the optical yield, and also to obtain the

(+)-enantiomer, we carried out the reduction in the presence of additives, such as allyl alcohol and allyl bromide [4]. We also tested some other allyl derivatives such as allyl amine, allyl acetate and allyl-*p*-toluenesulphonate, as additives in baker's yeast reductions, to see whether they had any effect. In addition, we carried out the reduction of the same substrate **1** with the micro-organism *Aspergillus niger*, which is reported to afford reductions of ketones with opposite stereochemistry with respect to yeast [6]. The results obtained from these reductions are reported in Tables 1 and 2.

As reported in a previous work dealing with yeast reductions of diketones [4], opposite and efficient stereochemical control in the reduction of **1** was observed when allyl alcohol and allyl bromide are added as additives to the yeast suspension in the presence or absence of glucose. In the presence of allyl alcohol we recovered the levorotatory hydroxyester **2** with enhanced enantiomeric excess (71–86% ee, Table 1); in turn, when reduction with yeast was carried out in the presence of allyl bromide, we recovered the dextrorotatary enantiomeric form of the hydroxyester **2** with 54–68% ee (Table 1). In each case the higher conversion is observed in the presence of glucose. Other allylic additives, such as allyl amine, allyl acetate and allyl-*p*-toluensulphonate, did not afford significant results: we recovered the levorotatory form of **2** with 24–67% ee (Table 1).

The reductions carried out with *Aspergillus niger* afforded only the levorotatory hydroxyester **2** in low conversion and with 23–68% ee, depending upon the culture medium and the time of incubation (Table 2).

Table 1
Results of the reduction of methyl 4-chloro-4,4-difluoro-3-oxobutanoate **1** with yeast and in the presence of additives. The reaction conditions refer to 1 mmol of substrate in 25 ml of distilled water

Yeast (g)	Glucose (g)	Additive ($g\ l^{-1}$)	Conv.% [a]	ee% [b]	Enantiomer
2.80	0.0	–	100	21	(−)
2.8	0.0	allyl alcohol (1.5)	79	79	(−)
2.8	0.0	allyl alcohol (3)	61	86	(−)
2.8	1.5	–	100	53	(−)
2.8	1.5	allyl alcohol (3)	100	71	(−)
2.8	1.5	allyl alcohol (6)	97	81	(−)
2.8	1.5	allyl amine (1.5)	100	67	(−)
2.8	1.5	allyl *p*-toluene-sulphonate (3)	91	24	(−)
2.8	1.5	allyl acetate (1.5)	100	59	(−)
2.8	1.5	allyl bromide (3)	41	68	(+)
5.6	0.0	–	100	20	(−)
5.6	0.0	allyl bromide (3)	68	54	(+)
5.6	3.0	–	100	65	(−)
5.6	3.0	allyl bromide (3)	99	54	(+)

[a] Conversion evaluated by GLC after 24 h of incubation, at 27–30°C.
[b] Enantiomeric excess evaluated on a chiral G-DEX 120 column.

Table 2
Results of the reduction of methyl 4-chloro-4,4-difluoro-3-oxobutanoate **1** with *Aspergillus niger*

Culture medium	Time (days) [a]	Conv.% [b]	ee% [c]	Enantiomer
Czapek	1	27	68	(−)
Czapek	4	51	23	(−)
MPG	1	19	43	(−)
MPG	4	26	20	(−)
Sabouraud	1	28	56	(−)
Sabouraud	4	36	31	(−)

[a] Time of incubation of micro-organism/substrate reaction mixture.
[b] Conversion evaluated by GLC.
[c] Enantiomeric excess evaluated on a chiral G-DEX 120 column.

Acknowledgements

Thanks are do to CNR, Rome, for financial support.

References

[1] G. Resnati, Tetrahedron, 49 (1993) 9385.
[2] E. Santaniello, P. Ferraboschi, P. Grisenti and A. Manzocchi, Chem. Rev., 92 (1992) 1071.
[3] K. Nakamura, in S. Servi (Ed.), Microbial Reagents in Organic Synthesis, NATO ASI Series, Kluwer, Netherlands, 1992, p. 389.
[4] A. Forni, I. Moretti, F. Prati and G. Torre, Tetrahedron, 50 (1994) 11995.
[5] C.W. Emmons, C.H. Binford, J.P. Utz and K.J. Kwon-Chung, Medical Mycology, Lea and Febiger, PA, 1977, p. 535.
[6] A. Fauve and H. Veschambre, J. Org. Chem., 53 (1988) 5215.