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SCIENCE DIRECT®
Tetrahedron: Asymmetry
Tetrahedron: *Asymmetry* 15 (2004) 1945–1947

Enantioselective hydrolysis of (*RS*)-isopropylideneglycerol acetate with *Kluyveromyces marxianus*

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Received 15 April 2004; accepted 11 May 2004

Abstract—The hydrolysis of (*RS*)-isopropylideneglycerol acetate with whole cells of the yeast *Kluyveromyces marxianus* is reported. The biotransformation furnished (*R*)-isopropylideneglycerol as major enantiomer with good enantioselectivity ($E = 28$) under optimised conditions. The reaction can be performed in an ultrafiltration-membrane reactor allowing for the obtainment of 19.2 g/L of enantiomerically pure (*R*)-isopropylideneglycerol acetate starting from 60 g/L of racemic mixture.

1. Introduction

The resolution of racemic 2,2-dimethyl-[1,3]dioxolan-4-methyl acetate (1,2-*O*-isopropylideneglycerol, IPG or solketal) esters is an easy method for obtaining both the enantiomers of IPG. Enantiomerically pure IPG is a valuable chiral building blocks, for example, in the synthesis of β-adrenoceptor antagonists, prostaglandins and leukotrienes.[1,2]

The enantioselective hydrolysis of (*RS*)-isopropylideneglycerol esters is difficult to achieve with commercially available enzymes,[3–6] while the best results have been obtained by hydrolysis of IPG caprylate and benzoate using esterases from *Bacillus* species: a carboxylesterase from *Bacillus coagulans* is able to enantioselectively hydrolyse IPG benzoate to produce (*S*)-IPG[7] and the same enantiomer is predominantly formed by a recently isolated esterase from *Bacillus subtilis* in the hydrolysis of IPG caprylate.[8] Enzymatic methods with preference for the hydrolysis of (*S*)-IPG esters have not yet been developed.

Herein we report the enantioselective hydrolysis of (*RS*)-IPG acetate with the easily cultivable yeast *Kluyveromyces marxianus*, which had shown in preliminary studies preference for the formation of (*R*)-IPG.[9] The kinetic resolution of (*RS*)-IPG acetate by enzymatic hydrolysis has some advantages since the substrate has a good solubility in water and its synthesis is cheap.

2. Results

The hydrolysis of (*RS*)-isopropylideneglycerol acetate was firstly studied using different strains of *Kluyveromyces marxianus* as biocatalysts. Hydrolysis was carried out using 3.0 g/L of racemic substrate and 35 g/L of dry yeast in 1/15 M phosphate buffer at pH 7.0, at the temperature of 30 °C. The ability to perform the rapid hydrolysis of IPG acetate was widespread among this yeast' species and the enzymatic activity was found to be mostly cell bound, with enantiomeric ratios in the range of 12–16 (Table 1).

K. marxianus CBS 1553 was used in further experiments aimed at the optimisation of the biotransformation. The yeast was lyophilised showing similar performances and maintained for six months at −20 °C with no significant loss of activity/enantioselectivity; therefore, lyophilised biomass from a single 10 L fermentation could be used throughout this study. Optimisation was performed following a Multisimplex experimental design:[10,11] type of growth medium, cell and substrate concentrations,

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doi:10.1016/j.tetasy.2004.05.010

Table 1. Hydrolysis of racemic IPG acetate catalysed by different strains of *Kluyveromyces marxianus*; results after 3 h

Strain	Ee % (*R*)-IPG acetate	Ee % (*R*)-IPG	Molar conv. (%)	*E*
CBS 397	48	82	37	16
CBS 607	53	76	41	12
CBS 834	68	74	48	15
CBS 1553	90	69	57	16
CBS 2231	55	78	41	13
CBS 2762	58	77	43	13
CBS 4857	51	81	39	15

temperature and pH of the biotransformation were chosen as response variables. Biomass concentrations ranging between 25–30 g/L (dry cells) used in tap water at pH 7.5 at 28 °C gave the highest enantioselective activity. Under these reaction conditions, different amounts of substrate were transformed (Table 2).

The highest *E* value was observed at the lowest substrate concentration, nevertheless the best compromise between overall enantioselectivity, reaction rate, yields and recovery of enantiomerically pure substrate was obtained using 3 g/L.

Repeated-batch experiments aimed at improving the productivity were firstly carried out with *Kluyveromces marxianus* CBS 1553. The biocatalyst was recycled by simply paper filtering or centrifuging the cells when the biotransformation had reached the desired conversion, but the process was complicated by the partial release of the carboxylesterase activity into the medium. Therefore, an ultrafiltration (cut-off 10,000 Da) membrane reactor was employed for semi-continuous operation for maintaining the cells and the released enzyme inside the reaction vessel. Ultrafiltration was applied when the enantiomeric excess of the substrate had reached 100%. Repeated-batch operation of the membrane reactor was maintained for 20 cycles, before decrease of the enzymatic activity could be observed; this operation allowed the recovery of 19.2 g/L of enantiomerically pure (*R*)-IPG acetate starting from 60 g/L of racemic mixture.

3. Conclusion

In conclusion, it was found that the carboxylesterase activity bound to the cells of *K. marxianus* is suited for the resolution of (*RS*)-IPG acetate, furnishing (*R*)-IPG with good yields. The stability of the biocatalyst is good and multigram production of the (*R*)-enantiomer can be obtained by using a simple membrane reactor.

4. Experimental

4.1. Microorganisms, growth and biotransformation conditions

Strains of *K. marxianus* were from CBS (Central Bureau voor Schimmelcultures, Baarn, Holland) and routinely maintained on malt extract (8 g L^{-1}, agar 15 g L^{-1}, pH 5.5). They were cultured in 2.0 L Erlenmeyer flasks containing 200 mL of malt broth pH 6.0 and incubated for 48 h at 28 °C on a reciprocal shaker (100 spm). *K. marxianus* CBS 1553 was employed in optimisation studies accomplished using cells from a 8 L fermenter containing 4.0 L of liquid medium for 48 h, pH 6.0, at 27 °C and agitation speed 100 rpm. The dry weights were determined after centrifugation of 100 mL of cultures, cells were washed with distilled water and dried at 110 °C for 24 h.

Biotransformations were carried out in 10 mL screw capped test tubes with cells suspended in 5 mL of different aqueous media. After 45 min of incubation, neat substrate was added and the incubation continued under magnetical stirring.

A stirred ultrafiltration cell (Model 8050, capacity 50 mL) containing 20 mL of biotransformation medium was employed as membrane reactor for repeated-batch biotransformation. The membrane had a cut-off of 10,000 Da. The reaction was stopped when the enantiomeric excess of the substrate was 100% by filtering the suspension under nitrogen pressure. The recovered filtrate was extracted with ethyl acetate and (*R*)-isopropylideneglycerol acetate was purified as described for the synthesis of the racemic substrate $[\alpha]_D^{20} = -8.3$ (*c* 1, $CHCl_3$).

4.2. 1,2-*O*-isopropylideneglycerol acetate

The synthesis of (*RS*)-isopropylideneglycerol acetate was carried out by adding acetic anhydride (12 mL) and pyridine (8 mL) to a solution of racemic 1,2-*O*-isopro-

Table 2. Hydrolysis of different amounts of racemic IPG acetate catalysed by *Kluyveromyces marxianus* CBS 1553

(*RS*)-IPG acetate		(*R*)-IPG acetate			Time (h)	*E*[a]
Initial concentration (g/L)	Conv. (%)[a]	Ee (%)	Yield (%)[b]	Recovery (g/L)[c]		
1	62	>99	76	0.30	3	28
3	62	>99	76	1.05	4	28
5	70	>99	60	1.32	15	15
8	75	>99	50	1.65	24	11
10	62	92	—	—	24	11

[a] Conversion and enantioselectivity factor (*E*) calculated from the ee of the substrate and the product.
[b] Calculated taking into account the conversion.
[c] After purification by flash column chromatography.

pylideneglycerol (8 g) in dry benzene (50 mL). After 24 h at room temperature, the reaction mixture was quenched with 150 mL of 5% $NaHCO_3$ solution and the product extracted with ethyl acetate. The organic extracts were dried over Na_2SO_4 and the solvent removed. The crude product was purified by flash chromatography (ethyl acetate/hexane 1/1) yielding 9.35 g of pure product. ^1H NMR ($CDCl_3$, 200 MHz): δ 1.38 (s, 3H, CH_3), 1.44 (s, 3H, CH_3), 2.12 (s, 3H, CH_3), 3.36–3.41 (m, 2H, CH_2), 4.01–4.11 (m, 2H, CH_2), 4.13–4.19 (m, 1H, CH).

4.3. Optimisation by sequential simplex method

The simplex optimisation method was based on sequential experimental trials guided by the systematic search strategies of the Multisimplex® 2.0 program (Multisimplex AB, Karlskrona, Sweden).[9] The starting experiments were selected with levels of each control variable (substrate concentration, pH, temperature and biomass concentration) within the following ranges: pH 4–8, temperature 20–50 °C, substrate concentration 1–5 g/L, biomass concentration 15–30 g/L. The control responses to be optimised were the molar conversion after 3 h and the corresponding enantioselectivity factor (E). Each experiment was carried out in triplicate.

4.4. Analytical methods

Alcohol and ester concentrations were determined by gas-chromatographic (GC) analysis on a Carlo Erba Fractovap GC gas chromatograph equipped with a hydrogen flame ionisation detector. The column (3×2000 mm) was packed with Carbowax 1540 (10% on Chromosorb 80–100 mesh). Samples (0.2 mL) were taken at intervals and added to an equal volume of an internal standard solution (1-hexanol 2 g/L) in water; the resulting solution was extracted with ethyl acetate and analysed. The enantiomeric composition of IPG acetate and IPG was determined by gas-chromatographic analysis using a chiral capillary column (diameter 0.25 mm, length 25 m, thickness 0.25 μ, DMePeBeta-CDX-PS086, MEGA, Legnano, Italy). The absolute configurations of IPG were determined by comparison with enantiomerically pure samples commercially available (Sigma–Aldrich).

References and notes

1. Jurczak, J.; Pikul, S.; Bauer, T. *Tetrahedron* **1986**, *42*, 447–488.
2. Xia, J.; Hui, Y. Z. *Tetrahedron: Asymmetry* **1997**, *8*, 3019–3021.
3. Hess, R.; Bornscheuer, U.; Capewell, A.; Scheper, T. *Enzyme Microb. Technol.* **1995**, *17*, 725–728.
4. Bianchi, D.; Borsetti, A.; Golini, P.; Cesti, P.; Pina, C. *Tetrahedron: Asymmetry* **1997**, *8*, 817–819.
5. Jaeger, K. E.; Schneidinger, B.; Rosenau, F.; Werner, M.; Lang, D.; Dijkstra, B. W.; Schimossek, K.; Zonta, A.; Reetz, M. T. *J. Mol. Catal. B* **1997**, *3*, 3–12.
6. Lorenz, P.; Liebeton, K.; Niehaus, F.; Eck, J. *Curr. Opin. Biotechnol.* **2002**, *13*, 572–577.
7. Molinari, F.; Brenna, O.; Valenti, M.; Aragozzini, F. *Enzyme Microb. Technol.* **1996**, *19*, 551–556.
8. Droge, M. J.; Bos, R.; Quax, W. J. *Eur. J. Biochem.* **2001**, *268*, 3332–3338.
9. Maconi, E.; Potenza, D.; Valenti, M.; Aragozzini, F. *Ann. Microbiol.* **1990**, *40*, 177–186.
10. Walters, F. H.; Parker, L. R.; Morgan, S. L.; Deming, S. N. *Sequential Simplex Optimization*; CRC: Boca Raton, 1991.
11. Molinari, F.; Villa, R.; Aragozzini, F.; Leon, R.; Prazeres, D. M. F. *Tetrahedron: Asymmetry* **1999**, *10*, 3003–3009.