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Tetrahedron: Asymmetry
Tetrahedron: *Asymmetry* 16 (2005) 3279–3282

A new chemoenzymatic synthesis of D-cloprostenol

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Received 18 August 2005; accepted 30 August 2005
Available online 28 September 2005

Abstract—A new chemoenzymatic synthesis of D-cloprostenol based on the biocatalytical resolution of *anti*-2-oxotricyclo[2.2.1.0]heptan-7-carboxylic acid **1** has been developed. The resolution was attempted by different approaches: esterification or reduction of the acid and hydrolysis or reduction of the corresponding esters. The most efficient method proved to be the reduction of the propyl esters of **1** catalysed by the yeast *Kluyveromyces marxianus*, which allowed for the recovery of the enantiomerically pure ester of *anti*-2-oxotricyclo[2.2.1.0]heptan-(*R*)-7-carboxylic acid (*R*)-**3** at 60% molar conversion of 3.0 g/l of racemic substrate acid under optimised conditions. *anti*-2-Oxotricyclo[2.2.1.0]heptan-(*R*)-7-carboxylic acid was obtained by alkaline hydrolysis and employed for the synthesis of D-cloprostenol.

1. Introduction

The synthesis of prostaglandins via bicyclo[2.2.1]heptane derivatives is a well-established approach.[1,2] *anti*-2-Oxotricyclo[2.2.1.0]heptan-7-carboxylic acid **1** is a useful chiral intermediate for prostaglandin synthesis and can easily be synthesised from norbornadiene as a racemic mixture,[3] but its synthetic utility is limited to the (7*R*)-stereoisomer. The resolution of (*RS*)-**1** can be achieved by precipitation with chiral amines, but the process is characterised by low yields.[4,5] The resolution can also be achieved by the enzymatic hydrolysis of the corresponding methyl ester with commercial lipases.[6,7]

Different biocatalytic approaches for the resolution of **1** or its esters (Scheme 1) have been investigated herein as an alternative to commercial enzymes. The resulting *anti*-2-oxotricyclo[2.2.1.0]heptan-(*R*)-7-carboxylic acid (*R*)-**1** was employed for the synthesis of D-cloprostenol, carried out by standard synthetic methods.

2. Results and discussion

The direct esterification of **1** was first investigated with ethanol, *n*-propanol and *n*-butanol using 22 commercial enzymes and 50 lyophilised microbial cells as biocatalyst in organic solvents.[8,9] Although different reaction conditions (temperature, reagent/biocatalyst ratio and organic solvent) were checked, the reaction rates always remained very sluggish. The best results were obtained after 7 days with lipase AH from *Pseudomonas cepacia* in toluene (20% molar conversion and $E = 8.0$) and with lyophilised mycelium of *Rhizopus oryzae* CBS 391.34 in *n*-heptane (15% molar conversion and $E = 8.5$) starting from 2.5 g/l of **1** and an equimolar amount of ethanol. Transesterification of the methyl ester of **1** with different alcohols was also evaluated, but no significant improvement in the yield or enantioselectivity was observed.

Enzymatic hydrolysis of the methyl ester of (*RS*)-**1** (R = Me) was investigated using various microorganisms previously selected in our laboratory for carboxylesterase activity.[10,11]

Whole cells of *Bacillus coagulans* NCIMB 9365[12] gave, under the optimal conditions (40 °C, phosphate buffer pH 6.5 and 30 g/l of biocatalyst), the highest enantioselectivity (Table 1).

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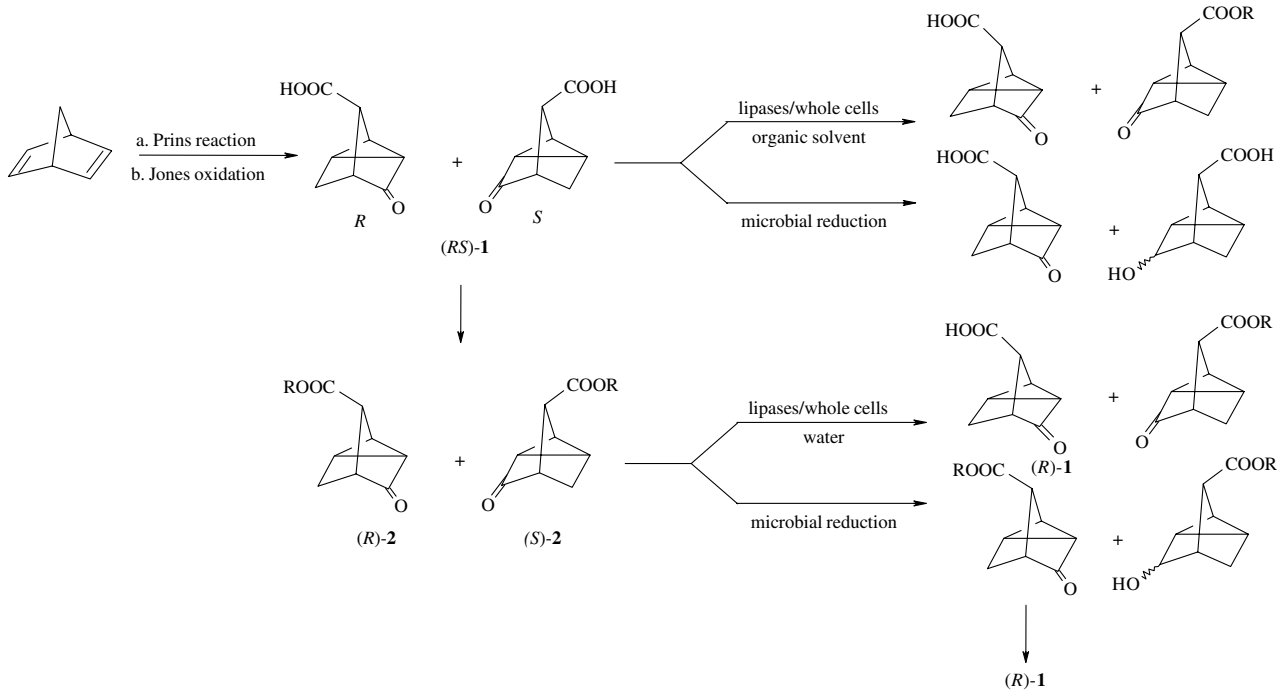
0957-4166/$ - see front matter © 2005 Published by Elsevier Ltd.
doi:10.1016/j.tetasy.2005.08.038



Scheme 1. Biocatalytical approaches for the resolution of *anti*-2-oxotricyclo[2.2.1.0]heptan-7-carboxylic acid (*RS*)-**1**.

Table 1. Hydrolysis of different esters of **1** with *Bacillus coagulans* NCIMB 9365

Ester	Molar conversion (%)	ee (%) of the remaining (*R*)-ester	*E*	Time (h)
Methyl	54	75	10	24
Ethyl	60	84	9	24
Propyl	63	>99	25	24
Butyl	68	>99	12	24
Pentyl	67	95	9	24
Cl-ethyl	65	93	9	3
Br-ethyl	59	70	3	3
Benzyl	74	70	4	8

The propyl ester (*RS*)-**2** (R = Pr) was hydrolysed with the highest enantioselectivity and the enantiomerically pure unreacted ester was recovered from the biotransformation mixture by filtration and extraction with ethyl acetate at an alkaline pH.

The biocatalytic reduction of the carbonyl group in **1** and its esters (*RS*)-**2** (R = methyl, propyl and butyl) was also investigated. A screening was performed with 70 yeasts from international collections belonging to different genera and species. *Kluyveromyces marxianus* CBS 2235 resulted to be the most enantioselective: optimisation of the reaction parameters (biocatalyst and substrate concentration, pH, temperature, type and concentration of co-substrate) showed that the use of 15 g/l of biocatalyst at 28 °C, in a phosphate buffer (pH 7.0, 0.1 M) in the presence of 25 g/L of glucose allowed for 60% conversion of 3.0 g/L of substrate within 90 min with >99% ee of the desired unreacted stereoisomer (Fig. 1). The use of *K. marxianus* for the enantioselective reduction of carbonyls has already been reported for the preparation of useful chiral intermediates.[13–17]

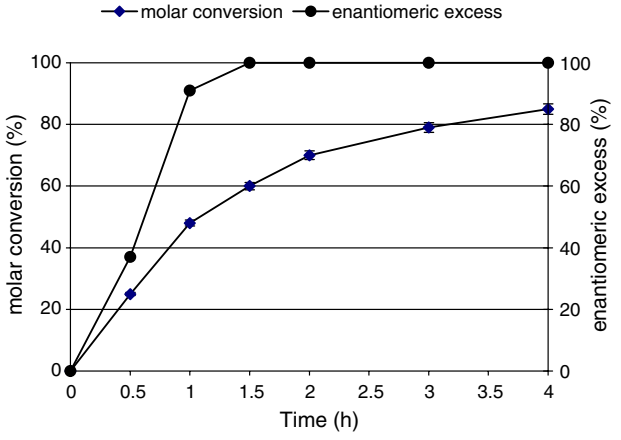


Figure 1. Molar conversion and enantiomeric excess of propyl ester (*RS*)-**2** (R = Pr) with *Kluyveromyces marxianus* CBS 2235; enantiomeric excess is referred to the unreacted propyl ester of *anti*-2-oxotricyclo[2.2.1.0]heptan-(*R*)-7-carboxylic acid (*R*)-**2** (R = Pr).

The reduction was also performed on a larger scale (50 L reactor) giving similar or improved results. The

crude extract, recovered after filtration of the biomass, extraction with ethyl acetate and solvent evaporation, was derivatised with a Girard reagent for easy separation of the alcohol produced. The enantiomerically pure ester was hydrolysed and used for next steps aimed at the production of D-cloprostenol.

3. Conclusion

In conclusion, the resolution of the propyl ester of *anti*-2-oxotricyclo[2.2.1.0]heptan-7-carboxylic acid (*RS*)-**2** (R = Pr) was efficiently achieved by the hydrolysis of the ester or by the reduction of the carbonyl group. In the latter case, although the reactive site is quite remote from the stereocentre involved in the resolution, the rigid structure allowed for sufficient stereodiscrimination. Stereoselective reduction with *Kluyveromyces marxianus* CBS 2235 showed a few technical advantages, such as inexpensive fermentation, high biomass production, easy scale-up and the simple separation of the alcohol by derivatisation as Girard reagent.

4. Experimental

4.1. General

Chemicals were of reagent grade and purchased form Fluka, Milano, Italy.

4.2. Microorganisms, media and culture conditions

The microorganisms were from CBS (Centraal Bureau voor Schimmelcultures, Baarn, The Netherlands), MIM (Microbiologia Industriale Milano, Italy), NCIMB (National Collection of Industrial and Marine Bacteria, Aberdeen, UK) and cultured as described before.[7] *Bacillus coagulans* NCIMB 9365 and *Kluyveromyces marxianus* CBS 2235 were cultured in 5.0 L fermenters: *Bacillus coagulans* was grown at 45 °C using CYSP broth agitation speed 100 rpm (casitone 15 g L^{-1}, yeast extract 5 g L^{-1}, soytone 3 g L^{-1}, peptone 2 g L^{-1}, $MgSO_4 \cdot 7H_2O$ 15 mg L^{-1}, $FeCl_3$ 115 mg L^{-1}, $MnCl_2$ 20 mg L^{-1} and pH 7.0), while *Kluyveromyces marxianus* with 1.0 L of malt broth pH 6.0 for 48 h, at 27 °C and agitation speed 100 rpm.

4.3. Synthesis of *anti*-2-oxotricyclo[2.2.1.0]heptan-7-carboxylic acid esters (*RS*)-2

Anti-2-oxotricyclo[2.2.1.0]heptan-7-carboxylic acid (*RS*)-**1** (2.0 g) was dissolved in dry CH_2Cl_2 (30 mL) and dimethylaminopyridine (DMAP, 150 mg) and the corresponding alcohol (0.9:1 molar ratio with respect to the acid) were added; the mixture was brought to 4 °C and dicyclohexylcarbodiimide (DCC, 2.45 g) was added and the reaction mixture stirred for 3 h at room temperature. The mixture was filtered and the filtrate evaporated at 35 °C under vacuum. The residue was purified by flash chromatography (*n*-hexane/ethyl acetate = 1:1) to give the desired ester with yields in the range of 80–95%.

4.4. Enzymatic esterification of *anti*-2-oxotricyclo-[2.2.1.0]heptan-(*RS*)-7-carboxylic acid (*RS*)-1

Anti-2-oxotricyclo[2.2.1.0]heptan-7-carboxylic acid (*RS*)-**1** (10.0 mg) was dissolved in organic solvent (5.0 mL) and dry biocatalyst (125 mg as lyophilised cells or dry enzymes) was added; the mixture was stirred at different temperatures for 7 days and the reaction followed by TLC (ethyl acetate/acetic acid = 98:2). After 7 days, the reaction mixture was filtered and the organic phase evaporated. The residue was purified by chromatography (*n*-hexane/ethyl acetate = 1:1 for recovering the formed ester product and then ethyl acetate/acetic acid = 98:2 for recovering the substrate). The enantiomeric composition of the methyl, *n*-propyl and *n*-butyl ester was determined by gas chromatographic analysis using a chiral capillary column (diameter 0.25 mm, length 25 m, thickness 0.25 μm, DMePeBeta-CDX-PS086, MEGA, Legnano, Italy) at 130 °C; the enantiomeric composition of **1** was determined by HPLC using a chiral column (Chiralcel OD, 4.6 × 250 mm, Daicel Chemical Industries Ltd., Tokio, Japan) mobile phase: *n*-hexane/2-propanol/HCOOH 90:10:1, flow 0.5 mL/min, temperature 28 °C, detection UV 280 nm.

4.5. Enzymatic hydrolysis of *anti*-2-oxotricyclo[2.2.1.0]-heptan-(*R*,*S*)-7-carboxylic acid esters (*RS*)-2

The biocatalyst (125 mg of whole cells or commercial enzyme) was added to 5 mL of phosphate buffer (1/15 M, pH 6.8) and the reaction started by the addition of the substrate (*RS*)-**2** (12.5 mg). The reaction was followed by chiral HPLC under the conditions already described. When the biotransformation had reached the desired degree of conversion and/or enantioselectivity, the suspension was centrifuged, and acidified with an aqueous solution of HCl (5%) and extracted with ethyl acetate. The extracts were washed with brine, dried over Na_2SO_4 and evaporated. The residue was purified by flash chromatography as described for the enzymatic esterification.

4.6. Screening for the reduction of methyl, propyl and butyl esters of *anti*-2-oxotricyclo[2.2.1.0]heptan-(*RS*)-7-carboxylic acid on small scale (*RS*)-2

The yeasts were centrifuged and suspended in 5 mL of phosphate buffer (1/15 M, pH 6.8) to reach a concentration of 15 mg/mL, glucose (125 mg) was added and the mixture stirred at 28 °C for 30 min. Racemic esters (*RS*)-**2** (R = methyl, propyl and butyl) (150 mg) were added and the mixture stirred at 28 °C. When the reaction had reached the desired conversion, the cells were separated by centrifugation and the aqueous phase extracted with ethyl acetate, dried over Na_2SO_4 and evaporated. The crude products were purified by flash chromatography ($CHCl_3$/ethyl acetate = 95:5) and analysed by gas chromatographic analysis using a chiral capillary column (diameter 0.25 mm, length 25 m, thickness 0.25 μm, DMePeBeta-CDX-PS086, MEGA, Legnano, Italy) at 130 °C.

4.7. Reduction of the propyl ester of *anti*-2-oxotricyclo-[2.2.1.0]heptan-(*RS*)-7-carboxylic acid (*RS*)-2 (R = Pr) with *Kluyveromyces marxianus* and recovery as Girard reagent

Kluyveromyces marxianus CBS 2235 (48 g dry weight) was suspended in 3.2 L of phosphate buffer (1/15 M, pH 6.8) containing 80.0 g of glucose and the mixture stirred for 45 min at 28 °C; (*RS*)-**2** (R = Pr) (8.0 g) was then added and the reaction followed by chiral GC. After 2 h, the enantiomeric excess of the substrate was 100% with 64–65% molar conversion and the reaction mixture was filtered; the aqueous phase was extracted with ethyl acetate, dried over Na_2SO_4 and evaporated giving 8.1 g of crude residue. The crude residue was dissolved in MeOH (240 mL) and acetic acid (12.8 mL) and Girard T reagent (16.0 g) then added. The mixture was refluxed for 15 h. The mixture was then cooled at 20 °C and NaOH (20% aqueous solution) was added until pH 7.0. MeOH was distilled under vacuum and the remaining solution was extracted with CH_2Cl_2. The organic extracts were washed with brine, dried over Na_2SO_4 and evaporated giving 2.0 g of enantiomerically pure propyl ester of *anti*-2-oxotricyclo[2.2.1.0]heptan-(*R*)-7-carboxylic acid (*R*)-**2** (R = Pr). $[\alpha]_D^{25} = +23.1$ (*c* 1.0 MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.98–1.02 (t, $J = 4.2$ Hz, 3H), δ 1.45–1.51 (dt, $J = 2.1$ Hz, 1H), δ 1.63–1.74 (m, 2H), δ 1.84–1.90 (dt, $J = 6.2$ Hz, 1H), δ 1.95–2.01 (dt, $J = 6.2$ Hz, 1H), δ 2.20–2.27 (m, 1H), δ 2.38–2.45 (m, 1H), δ 3.01 (bt, 1H), δ 4.05 (t, $J = 4.2$ Hz, 2H).

4.8. Hydrolysis of the propyl ester of *anti*-2-oxotricyclo-[2.2.1.0]heptan-(*R*)-7-carboxylic acid (R)-2 (R = Pr)

The propyl ester (*R*)-**2** (R = Pr) (690 mg) is added to a solution composed with H_2O (2.1 mL), MeOH (2.1 mL), *tert*-butyl methyl ether (2.1 mL), NaOH (0.30 g) and the mixture is left under stirring at room temperature for 18 h. When the reaction was over, pH was brought to pH 1.0 with an aqueous solution of HCl (5%) and MeOH evaporated under reduced pressure. The aqueous phase was extracted with isopropyl acetate, the organic extracts were washed with brine, dried over Na_2SO_4 and evaporated giving 490 mg of crude residue which was crystallised using isopropyl ether furnishing 410 mg of pure *anti*-2-oxotricyclo[2.2.1.0]heptan-(*R*)-7-carboxylic acid (*R*)-**1**. $[\alpha]_D^{25} = +93.0$ (*c* 1.0 dioxane), $[\alpha]_D^{25} = +81.1$ (*c* 1.0 MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.50 (t, $J = 5.9$ Hz, 1H), δ 1.92 (d, $J = 10.8$ Hz, 1H), δ 1.98 (d, $J = 10.8$ Hz, 1H), δ 2.15 (s, 1H), δ 2.16 (t, $J = 5.9$ Hz, 1H), δ 2.40 (t, $J = 5.9$ Hz, 1H), δ 3.04 (s, 1H). HRMS calculated for CHO 170 (M^+).

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