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A new chemoenzymatic synthesis of D-cloprostenol

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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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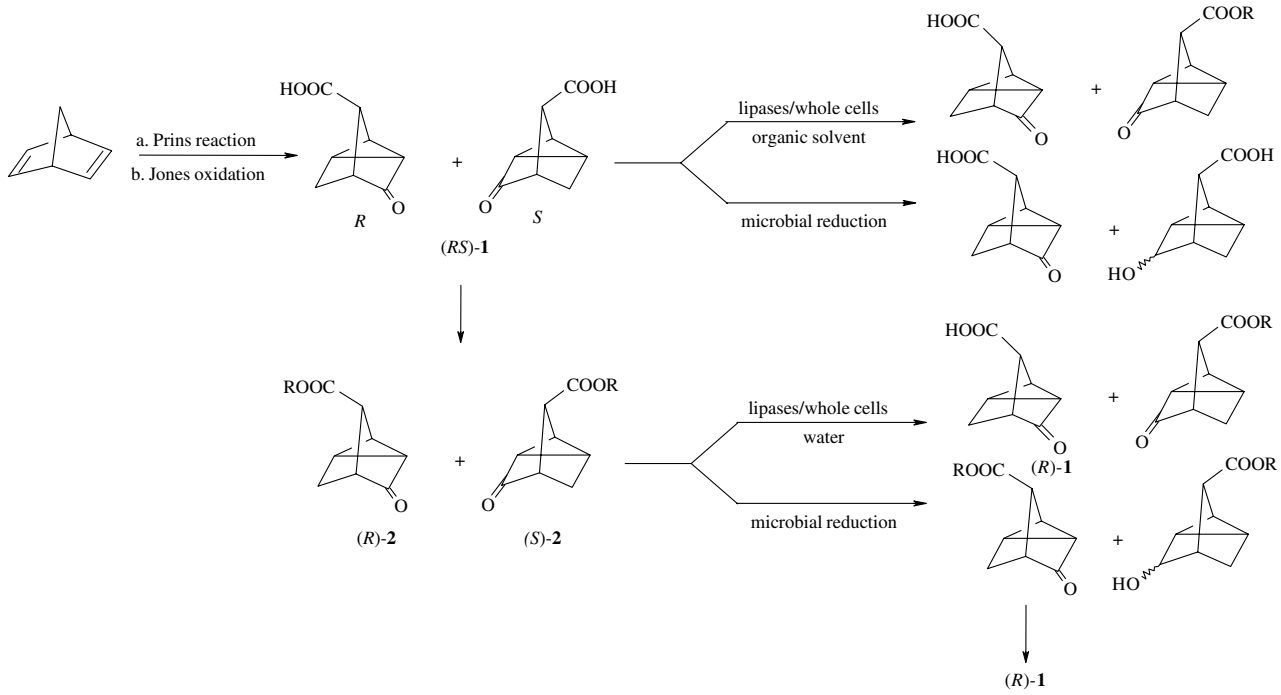

Scheme 1. Biocatalytical approaches for the resolution of *anti*-2-oxotricyclo[2.2.1.0]heptan-7-carboxylic acid (*RS*)-**1**.

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]

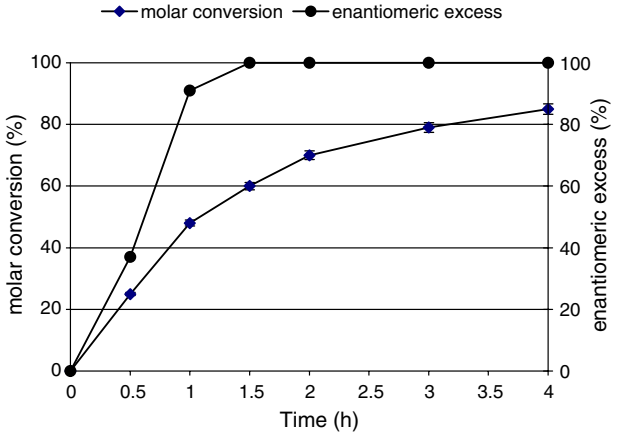

Figure 1. Molar conversion and enantiomeric excess of propyl ester (*RS*)-**2** (R = Pr) with *Kluyveromyces marxianus* CBS 2235; enantiomeric excess is referred to the unreacted propyl ester of *anti*-2-oxotricyclo[2.2.1.0]heptan-(*R*)-7-carboxylic acid (*R*)-**2** (R = Pr).

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