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A practical and scalable process for 4-(*R*)-hydroxycyclopent-2-en-1-(*S*)-acetate by desymmetrization of *meso*-cyclopent-2-en-1,4-diacetate catalyzed by *Trichosporon beigelii* (NCIM 3326), a cheap biocatalyst†

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Abstract

Various yeast and fungal cultures from NCIM, NCL, Pune, India were screened for the hydrolysis of *meso*-cyclopent-2-en-1,4-diacetate **2** to 4-(*R*)-hydroxycyclopen-2-en-1-(*S*)-acetate **1** to provide a cheaper and more effective alternative to PLE which is currently being used for the conversion. Yeast cultures of *Trichosporon* species were identified as having a pro-*R* preference in the hydrolysis of **2**; but the enantioselectivity was poor. Hence detailed medium-engineering investigations were made for the hydrolysis of **2** to **1** using a culture of *Trichosporon beigelii* (NCIM 3326) as catalyst. Addition of 10% v/v ethanol was found to enhance the enantioselectivity of the enzyme, affording **1** of 85% optical purity (op) in 83% yield. Further exploration of inherent consecutive kinetic resolutions to the desymmetrization afforded **1** of >98% op in 74% chemical yield.

1. Introduction

Over the past decade, chemoenzymatic methods using microorganisms and isolated enzymes as natural chiral catalysts have emerged as extremely efficient tools for the synthesis of enantiomerically pure materials on the large scale.[1] Various large-scale processes based on such methods have become important sources of chiral products.[2] We have been interested in the development of economically viable large-scale processes for prostaglandins and their intermediates due to their varied biological activities.

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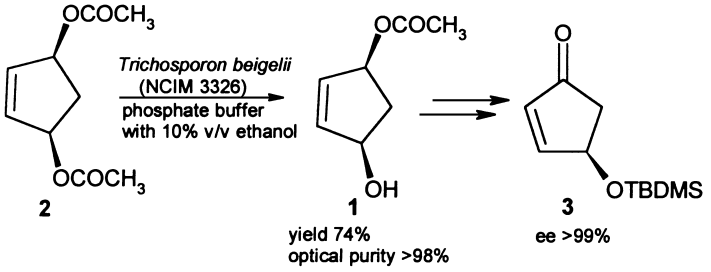
4-(*R*)-Hydroxycyclopent-2-en-1-(*S*)-acetate **1** is an important intermediate in the synthesis of cyclopentanoid natural products[3a] e.g. prostaglandins, prostacyclins, thromboxanes and recently in the synthesis of some anti-HIV drugs.[3b] This has resulted in various approaches for its preparation.[4] Enzyme viz. lipase/esterase-catalyzed desymmetrization of *meso*-cyclpent-2-en-1,4-diol by transesterification[5] or enzymatic hydrolysis of its *meso*-diacetate **2**,[6] or kinetic resolution of monoprotected cyclopentenediol[7] appear to be the methods of choice in the preparation of enantiomerically pure **1**. The latter method, viz. kinetic resolution, suffers from the drawback of throwing half of the material away; whereas use of the desymmetrization method allows 100% yield to be attained theoretically. In the case of the desymmerization of **2** through enzymatic hydrolysis in aqueous medium, most of the efficient enzymes reported, with the exception of porcine liver esterase (PLE),[6c] show a pro-*S* preference yielding *ent*-**1** in high enantiomeric excess. It is possible to get the desired (4*R*)-hydroxy configuration through manipulation of this (4*S*)-hydroxy enantiomer (*meso*-trick).[3b,8] But this adds to the steps and involves use of diethylaluminium chloride or DIBALH which are costly and hazardous, hence not recommended for large scale. Therefore, direct desymmetrization of **2** to **1** through aqueous hydrolysis is very desirable.

PLE having a pro-*R* preference for the hydrolysis of **2** yields **1** on hydrolysis and is currently used as the method of preparation. However, PLE is expensive and also gives **1** of only 81% ee which has to be recrystallized to enhance the ee to >96%, resulting in overall low yields.[6c] Hence replacement by other cheaper enzymes of microbial origin is required for the economization of the process.

The large collection of over 300 yeast and fungal cultures at NCIM, India offered us a great opportunity to explore the possibility of hunting a microbial culture of desired selectivity for the conversion of **2** to **1**. Herein we report our successful efforts in finding a yeast culture having the desired pro-*R* selectivity for the conversion of **2** to **1** through aqueous hydrolysis wherein the desired level of efficiency of the conversion in terms of enantiopurity and yield of the product was achieved through medium-engineering.

2. Results and discussion



Various cultures grown on oil media were tested for hydrolytic activity on **2** in 0.1 M phosphate buffer. Reactions were monitored by TLC. Where monoacetate was the hydrolysis product, its configuration and optical purity (op) were determined from the sign and value of the optical rotation, respectively. Most of the cultures having hydrolytic activity for **2** were pro-*S* selective affording *ent*-**1** in varying op. Only a few cultures belonging to the *Trichosporon* species exhibited the pro-*R* selectivity; however, the optical purities obtained were poor (Table 1). Since we could not find any culture having high enantioselectivity for the pro-*R* acetate, we decided to

optimize the conversion using this poorly selective *Trichosporon* species of good hydrolytic activity with a pro-*R* preference.

Table 1
Results of preliminary screening[a]

No.	Culture Name	NCIM No.	Yield of 1 %	$[\alpha]_D$ of 1[b]	Optical purity of 1 %
1.	*Trichosporon beigelii*	3326	88	-20.2	29.1
2.	*Trichosporon sp.*	3369	75	-5.8	8.4
3.	*Trichosporon sp.*	3382	78	-5.5	7.9
4.	*Trichosporon beigeii*	3404	76	-15.6	22.5
5.	*Trichosporon captatum*	3412	78	-16.4	23.7

a: **2** (0.050g, 0.27mmol) was reacted with wet cell cultures in 0.1 M phosphate buffer (5ml) for 18 hr at 30°C on orbital shaker. *b*: Rotations were measured in chloroform using 1% concentration. Lit.[11] $[\alpha]_D$ = -69.3 (c 1, chloroform).

Enhancement of enzyme efficiency through medium engineering, i.e. optimization of the solvent system, has been well documented in several cases.[9] Addition of co-solvents, such as alcohols, acetone, DMF, DMSO, etc. in small percentages are reported to have beneficial effects on hydrolytic reactions. *Trichosporon beigelii* (NCIM 3326) was considered as the best candidate for selectivity enhancement studies as it showed the best results (88% yield, 29% op. Table 1) in preliminary screening studies. Reactions were carried out by adding various water-miscible co-solvents in 10% v/v in buffer and the results are presented in Tables 2–4. The above study indicates a strong effect of ethanol on the selectivity of the whole cell culture NCIM 3326. Thus, by using 10% ethanol in solvent system, the op of product **1** shot up from 29.1 to 82.6%. But

Table 2
Medium-engineering effect on the hydrolysis of **2** catalyzed by NCIM 3326[a]

No	Co-solvent	Yield of 1 %	$[\alpha]_D$ of 1	Optical purity of 1 %
1.	Methanol	76	-39.5	57.0
2.	Ethanol	83	-57.2	82.6
3.	2-Propanol	79	-42.9	61.9
4.	1-Butanol	81	-14.4	20.8
5.	Acetone	84	-45.3	65.4
6.	Dimethylsulphoxide	77	-21.4	30.9
7.	Dimethylformamide	75	-34.9	50.4

a: All the reactions were carried out at 30 °C on orbital shaker for 18 hr using 1% concentration of substrate **2** in 0.1 M phosphate buffer (pH 7) containing 10% v/v of co-solvent.

Table 3
Effect of variation of ethanol concentration on the hydrolysis of **2** catalyzed by NCIM 3326

No.	Ethanol concentration %	Yield of 1 %	$[\alpha]_D$ of 1	Optical purity of 1 %
1.	4	81	-48.6	70.1
2.	10	83	-57.2	82.6
3.	20	79	-54.5	78.6
4.	50	No reaction	-	-
5.	80	No reaction	-	-

Table 4
Effect of 10% v/v ethanol in buffer media on the hydrolysis of **2** catalyzed by cultures from *Trichosporon* species

No.	Culture (NCIM No.)	Yield of 1 %	$[\alpha]_D$ of 1	Optical purity of 1 %
1.	3326	83	-57.2	82.6
2.	3369	78	-54.4	78.6
3.	3382	75	-45.3	65.4
4.	3404	74	-59.5	85.9
5.	3412	79	-53.5	77.3

high concentrations of ethanol (> 50%) were inhibitory for the reaction. Similar effects were observed with the cultures belonging to the same species (Table 4).

Even though the enantiopurity of **1** can be enhanced from 85 to > 98% by low temperature crystallization, which is a tedious process, the yields became low (65%). Sih et al. have demonstrated the importance of an inherent consecutive kinetic resolution step[6c] in enhancing the enantiomeric excess of chiral species obtained during enantioselective hydrolyses of *meso*-diesters. In the case studied, desymmetrization produces a mixture of **1** and *ent*-**1**, enriched in **1**. This mixture would further undergo kinetic resolution catalyzed by the same enzyme system. Here an enzyme having pro-*R* selectivity would preferentially catalyze hydrolysis of *ent*-**1** to the corresponding diol, thereby enhancing the optical purity of the remaining monoacetate **1**. This was demonstrated by carrying out the hydrolysis reaction for various time intervals and by checking the yield and optical purity of product **1** (Table 5). Thus, at 26 h product **1** was obtained in > 98% optical purity with 74% isolated yield.

Table 5
Effect of reaction time on the hydrolysis of **2** catalyzed by NCIM 3326 in 0.1 M (pH 7) buffer with 10% v/v ethanol

No.	Reaction time hr	Yield of 1 %	$[\alpha]_D$ of 1	Optical purity of 1 %
1.	15	85	-56.0	80.8
2.	18	83	-57.2	82.6
3.	20	80	-58.9	85.0
4.	26	74	-69.0	>98.0

Before scaling-up the process, it was necessary to optimize the substrate concentration tolerated by the enzyme. We found that a substrate concentration of 3.33% w/v was well tolerated by enzyme whereas at concentrations above this enzyme inhibition was observed. Thus, the process was successfully scaled-up to the 100 g scale using 3.33% substrate concentration and product **1** of greater than 98% optical purity was isolated in 74% yield. It was further converted to 4-(*R*)-*t*-butyldimethylsilyloxycyclopent-2-en-1-one **3** by known chemical transformations[3b,8] and its ee was determined to be > 99% by chiral HPLC.

Thus, our study has demonstrated *Trichosporon beigelii* (NCIM 3326),[10] a new very effective biocatalyst, as a significantly cheaper alternative to PLE for hydrolysis of *meso*-diacetate **2** to the expensive intermediate **1** in excellent optical purity. The study has also demonstrated the importance of medium-engineering approach for optimization of enzymatic process for an important chiral drug intermediate which can be scaled up.

3. Experimental

3.1. General

Optical rotations were recorded on a Jasco Dip-181 digital polarimeter using sodium vapor lamp. Optical purities (op) were determined by comparing the specific rotation value $[\alpha]_D$ with the literature values. All the reagents were of LR quality and were used without further purification.

3.2. General procedure for cell biomass preparation (whole cell enzyme)

The inoculum (5–10 ml) was developed by growing the microorganisms in a medium containing malt extract (0.3%), glucose (1%), yeast extract (0.3%) and peptone (0.5%) at pH 6.6–7.0 for 48 h with shaking at 150–180 rpm. This inoculum was transferred to medium containing $K_2H_2PO_4$ (0.2%), yeast extract (0.1%), peptone (0.5%), KCl (0.05%), $NaNO_3$ (0.05%), $MgSO_4 \cdot 7H_2O$ (0.05%), olive oil (1%) at pH 5.5 and incubated at 28–30°C for 36–48 h on rotary shaker (180–200 rpm). The grown cells were separated by centrifugation and the wet biomass was used for the reaction.

3.3. General procedure for enantioselective hydrolysis of meso-cyclopent-2-en-1,4-diacetate **2** to 4-(R)-hydroxycycloent-2-en-1-(S)-acetate **1**

meso-Diacetate **2** (100 g, 0.543 mol) was dissolved in ethanol (300 ml) in a 5 l three-necked round-bottomed flask equipped with an overhead stirrer, pH electrode and a dropping funnel. To the solution 0.1 M sodium phosphate buffer (pH 7, 2.7 l) was added and the mixture stirred vigorously using the overhead stirrer. To the stirred reaction mixture wet biomass (50 g) of *Trichosporon beigelii* (NCIM 3326) was added with a small amount of buffer. The whole mixture was stirred vigorously to yield a uniform emulsion. The pH of the reaction was monitored regularly and was maintained at 7 by adding 1 M aqueous sodium hydroxide solution through a dropping funnel. Progress of the reaction was monitored by TLC (30% ethyl acetate in pet. ether). Reaction was continued for 26 h. Then the reaction mixture was filtered through a Celite bed and the filtrate was extracted with ethyl acetate (3×2 l). The organic extracts were combined and washed with brine. The aqueous layer was separated and the organic layer was dried on anhydrous sodium sulfate. The solvent was removed on a rotary evaporator and dried at high vacuum. Product **1** was obtained as white crystalline needles. It was melted on a water bath and was stirred vigorously with pet. ether. The mixture was allowed to stand at room temperature for a few hours. Product **1** separated as fine crystalline needles. Pet. ether was decanted off and washing with pet. ether was repeated once again. These washings removed any unreacted diacetate **2** and other non-polar impurities. Yield of **1** was 57.40 g (74%). $[\alpha]_D = -68.9$ (*c* 1, $CHCl_3$), lit.[11] $[\alpha]_D = -69.3$ (*c* 1, chloroform) optical purity >98%.

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