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# A biocatalytic resolution of chiral ketals, intermediates in the synthesis of azole drugs

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**Abstract**

Crude lipases are used for the resolution of racemic ketals advanced intermediates in the synthesis of *cis*-terconazole and *cis*-ketoconazole. Lipase from *Aspergillus niger* allows to obtain the (2*R*, 4*R*)-enantiomer of the imidazole-substituted ketal in good ee at low conversion. The addition of adsorbing resins improves the efficiency to interesting ee values for practical applications. The compound is transformed into (2*R*, 4*S*)-terconazole. The survived ester gives access to the other enantiomer. © 2001 Elsevier Science B.V. All rights reserved.

*Keywords:* Terconazole; Ketoconazole; Lipase; Resolution

## 1. Introduction

Terconazole **3a** and ketoconazole **3b** are very potent antifungal agents currently marketed as racemates [1]. They are structurally related differing only for the substituent in the piperazine ring. Their pharmacological activity is associated with the inhibition of cytochrome *P*-450 enzymes involved in steroid biosynthesis. *Cis*-stereoisomers have most interesting biological properties. Differences among *cis*-enantiomers in ketoconazole have been detected [2], while similar studies concerning *cis*-terconazole activity have not been reported. Both enantiomers of the *cis*-stereoisomers of the two compounds have been prepared by total synthesis starting with traditional C-2 chiral synthons namely glycidol and solketal [2–4]. The synthesis currently in use for the racemates of the two compounds is depicted in Scheme 1. None of the two chiral synthons used in the mentioned synthesis of the enantiomerically pure compounds is ideal for the proposed synthetic scheme since during ketalization two stereoisomers are formed requiring separation. There is a current interest in the preparation of both enantiomers of racemic drugs with the intent to test the pharmacological activity of both enantiomers and eventually shift the production to the enantiomer bearing the more advantageous activity/toxicity ratio [5].

To this end, direct resolution of intermediate **rac5** or **6** looks interesting since: it gives a common intermediate for the preparation of both *cis*-imidazole derivatives, it enters directly into the actual synthetic scheme for the racemate.

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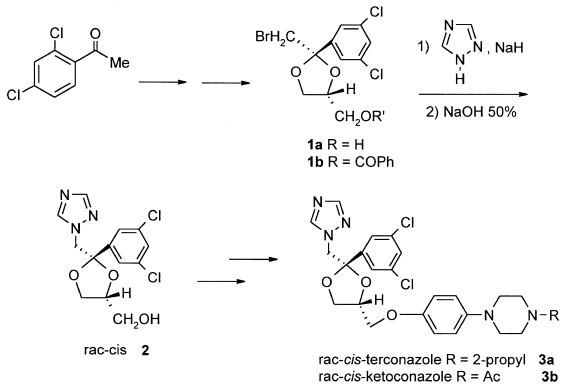



Scheme 1. Reaction scheme for production of *cis*-rac-**3a** and **3b**.

We have previously shown that esters of ketals of type **4** are good substrates for a number of enzymes, giving after hydrolysis the corresponding primary alcohols with good-medium ee [6]. We considered phenylacetates as substrates for penicillin G-amidase (PGA), phenyl propionates as candidates for α-chymotripsin (α-chy) hydrolysis and butyrates as possible substrates of lipases. In all cases, enantioselection was obtained, but penG acylase catalysed hydrolysis of the phenylacetate gave the best results (Scheme 2).

A comparison of structures **4** with **5** and **6** would suggest that enzymatic hydrolysis should occur on the latter substrates with similar outcome. The effect of the presence of the two bulky groups X–Y in **5** and **6**, if any, is expected to increase differentiation between the two enantiomeric forms and hence improve resolution [7]. We have therefore prepared esters **5** and **6a**–**c** and submitted to hydrolysis with the above-mentioned enzymes in order to secure the enantiomerically pure intermediates **1** and **2** for the synthesis of **3**.

## 2. Materials and methods

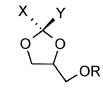
GC chiral analysis were performed on a DANI 8610 with a FID detector, fitted with a glass capillary column, Megadex DACTBSβ-cdx (Mega, Legnano, Italy), 25 mt × 0.25 mm i.d., film thickness 0.25 μm. HPLC were recorded on a Merck Hitachi L6000 equipped with L4000 UV detector and D2500 integrator. LiChroSpherSi60 and CHIRACEL OD were used as columns. $[\alpha]_{20}^{D}$ were recorded with a Propol automatic digital polarimeter. Immobilised PenG amidase was from Recordati (Italy). Other enzymes were from SIGMA. Compounds **1** and **2** were a gift from BIOINDUSTRIA (Novi Ligure, Italy). Adsorbing resins were from Mitsubishi and a gift of Resindion (Milano, Italy).

### *2.1. (2RS,4RS)-cis-2-(bromomethyl)-2-(2,4-dichlorophenyl)-4-phenylacetyloxy-1,3-dioxolane (**5a**)*

A total of 3 g of the alcohol **1a** was dissolved in 6 ml of anhydrous pyridine, while 2.7 g of phenylacetyl chloride in 2 ml of $CH_2Cl_2$ were added at O°C. The mixture was stirred at room temperature for 1 h, treated with 5 ml of a cold solution of 5% sodium bisulphite, 5 ml of water and 5 ml of a saturated $NaHCO_3$ solution. The organic phase was dried over anhydrous $Na_2SO_4$ and the solvent removed under reduced pressure. The crude oil was purified by flash-chromatography (*n*-hexane:2-propanol = 80:20) giving 2.6 g of **5a** (65%).

### *2.2. (2RS,4RS)-cis-2-(bromomethyl)-2-(2,4-dichlorophenyl)-4-butiryloxy-1,3-dioxolane (**5b**)*

A total of 2 g of the alcohol **1a** was dissolved in 4 ml of anhydrous pyridine,while 880 mg of butiryl chloride in 2 ml of $CH_2Cl_2$ were added at O°C. The mixture was stirred at room temperature for 1 h, treated with 5 ml of a cold solution of 5% sodium bisulphite, 5 ml of water and 5 ml of a saturated $NaHCO_3$ solution. The organic phase was dried over anhydrous $Na_2SO_4$ and the solvent removed under reduced pressure. The crude oil was purified by




**4** X = Y = $CH_3$
**5** X = $CH_2Br$ Y = 2,4-dichlorophenyl
**6** X = $CH_2$-(1,2,4)-triazolyl Y = 2,4-dichlorophenyl;
**a**) R = $PhCH_2CO$ **b**) R = nPrCO
**c**) R = $PhCH_2CH_2CO$

Scheme 2. Ketals as substrates for resolution with hydrolytic enzymes.

flash-chromatography (*n*-hexane:2-propanol = 80:20) giving 1.75 g of **5b** (72%).

### 2.3. (2RS,4RS)-cis-2-(bromomethyl)-2-(2,4-dichlorophenyl)-4-phenylpropionyloxy-1,3-dioxolane (5c)

A total of 5 g of the alcohol **1a** was dissolved in 8 ml of anhydrous pyridine, while 4.9 g of phenylpropionyl chloride in 5 ml of $CH_2Cl_2$ were added at O°C. The mixture was stirred at room temperature for 1 h, treated with 5 ml of a cold solution of 5% sodium bisulphite, 5 ml of water and 5 ml of a saturated $NaHCO_3$ solution. The organic phase was dried over anhydrous $Na_2SO_4$ and the solvent removed under reduced pressure. The crude oil was purified by flash-chromatography (*n*-hexane:2-propanol = 80:20) giving 4.3 g of **5c** (62%).

### 2.4. (2RS,4RS)-cis-2-(1,2,4-triazol-1-ylmethyl)-2-(2,4-dichlorophenyl)-4-phenylacetyloxy-1,3-dioxolane (6a)

A total of 1 g of the alcohol **2** was dissolved in 3 ml of anhydrous pyridine, while 700 mg of phenylacetyl chloride in 1 ml of $CH_2Cl_2$ were added at O°C. The mixture was stirred at room temperature for 1 h, treated with 5 ml of a cold solution of 5% sodium bisulphite, 5 ml of water and 5 ml of a saturated $NaHCO_3$ solution. The organic phase was dried over anhydrous $Na_2SO_4$ and the solvent removed under reduced pressure. The crude oil was purified by flash-chromatography (*n*-hexane:2-propanol = 80:20) giving 960 mg of **6a** (70%).

### 2.5. (2RS,4RS)-cis-2-(1,2,4-triazol-1-ylmethyl)-2-(2,4-dichlorophenyl)-4-butiryloxy-1,3-dioxolane (6b)

A total of 1 g of the alcohol **2** was dissolved in 3 ml of anhydrous pyridine, while 480 mg of butiryl chloride in 1 ml of $CH_2Cl_2$ were added at O°C. The mixture was stirred at room temperature for 1 h, treated with 5 ml of a cold solution of 5% sodium bisulphite, 5 ml of water and 5 ml of a saturated $NaHCO_3$ solution. The organic phase was dried over anhydrous $Na_2SO_4$ and the solvent removed under reduced pressure. The crude oil was purified by flash-chromatography (*n*-hexane:2-propanol = 80:20) giving 790 mg of **6b** (65%).

### 2.6. (2RS,4RS)-cis-2-(1,2,4-triazol-1-ylmethyl)-2-(2,4-dichlorophenyl)-4-phenylpropionyloxy-1,3-dioxolane (6c)

A total of 1 g of the alcohol **2** was dissolved in 3 ml of anhydrous pyridine, while 780 mg of phenylpropionyl chloride in 2 ml of $CH_2Cl_2$ were added at O°C. The mixture was stirred at room temperature for 1 h, treated with 5 ml of a cold solution of 5% sodium bisulphite, 5 ml of water and 5 ml of a saturated $NaHCO_3$ solution. The organic phase was dried over anhydrous $Na_2SO_4$ and the solvent removed under reduced pressure. The crude oil was purified by flash-chromatography (*n*-hexane:2-propanol = 80:20) giving 1.27 g of **6c** (90%).

### 2.7. Substrates hydrolysis with immobilised PenG acylase in aqueous media

A total of 60 mg of immobilised PenG acylase were suspended in 10 ml of $H_2O$ at pH 7.5 and 60 mg of ester **6a** or **5a** in 1 ml of acetonitrile were added. The reaction course was followed by titration of the developing acidity with NaOH 0.1 M until complete consumption of the starting material (3 h). Aliquots were withdrawn at regular time intervals and the enantiomeric composition of reactants and products was directly evaluated by chiral HPLC on a Chiralcel OD, *n*-hexane:2-propanol: 1:1, 0.5 ml/min.

### 2.8. Substrates hydrolysis with α-chymotripsin in aqueous media

A total of 50 mg of α-chymotripsin were dissolved in 10 ml of $H_2O$ at pH 7.5 and 100 mg of ester **6c** or **5c** in 1 ml of acetonitrile were added. The reaction course was followed by titration of the developing acidity with NaOH 0.1 M until complete consumption of the starting material (4 h). Aliquots were withdrawn at regular time intervals and the enantiomeric composition of reactants and products was directly evaluated by chiral HPLC on a Chiralcel OD column, *n*-hexane:2-propanol = 1:1, 0.5 ml/min.

Table 1

| Lipase | Amount (mg) |
|---|---|
| *Candida rugosa* | 5 |
| *Candida lypolitica* | 15 |
| *Aspergillus niger* | 15 |
| *Mucor javanicus* | 15 |
| PLE | 10 |
| PPL | 10 |

### *2.9. Substrates hydrolysis with lipase in aqueous media*

Hydrolytic enzymes used and their relative amount are indicated in Table 1.

The enzyme was dissolved in 1 ml of phosphate buffer 0.05 M, pH 7. 20 mg of esters **6b** or **5b** in 0.1 ml of acetonitrile were added. The reaction course was followed by titration of the developing acidity with NaOH 0.1 M until complete consumption of the starting material (4 h). Aliquots were withdrawn at regular time intervals and the enantiomeric composition of reactants and products was directly evaluated by chiral HPLC on a Chiralcel OD, *n*-hexane:2-propanol: 1:1, 0.5 ml/min for reaction mixtures containing **6**. The bromo containing esters were analysed on a system of 2 columns in series: LiChrospherSi 60 and Chiracel OD *n*-hexane:2-propanol: 1:1, 0.5 ml/min.

### *2.10. Hydrolysis by A. niger lipase of **6b** immobilised on a hydrophobic resin*

A total of 15 mg of *A. niger* lipase was dissolved in 1 ml of phosphate buffer 0.05 M, pH 7. 20 mg of esters **6b** were dissolved in methylene chloride and adsorbed by solvent evaporation on 20 mg of Sepabeads SP207 and the mixture stirred on a vibromax apparate in a test tube. The reaction course was followed by titration of the developing acidity with NaOH 0.1 M until complete consumption of the starting material (4 h). Aliquots were withdrawn at regular time intervals and the enantiomeric composition of reactants and products was directly evaluated by chiral HPLC on a Chiralcel OD, *n*-hexane:2-propanol: 1:1, 0.5 ml/min for reaction mixtures containing **6**. The bromo containing esters were analysed on a system of 2 columns in series: LiChrospherSi 60 and Chiracel OD *n*-hexane:2-propanol: 1:1, 0.5 ml/min.

### *2.11. Substrates hydrolysis in water saturated organic solvents*

Compounds **6a–c** (20 mg) were dissolved in 1 ml of water saturated solvent (toluene, methylene chloride, MTBE, 2-prOH, $Et_2O$). To the solution, 5–20 mg of the appropriate enzyme were added. The mixture was stirred on vibromax apparate. Aliquots were withdrawn and analysed by HPLC as previously described.

## 3. Results and discussion

Compound **5b** was submitted to lipase catalysed hydrolysis in a water suspension or in a water cosolvent solution. Hydrolysis rate with several enzymes was relatively rapid approaching 50% in less than 30 min for the enzymes form *C lypolitica*, *M javanicus* and PLE. However, the ee of the alcohol **1a** was completely disappointing. Transesterification reactions in organic solvents with several alcohols as donor behaved similarly giving products of low ee (Fig. 1). Compounds **5a** and **5c** were prepared and hydrolysed with penG acylase and α-chy, respectively. The enantiomeric excess of products was also in this case surprisingly low (50%). These data are lower than expected from the previous experience on

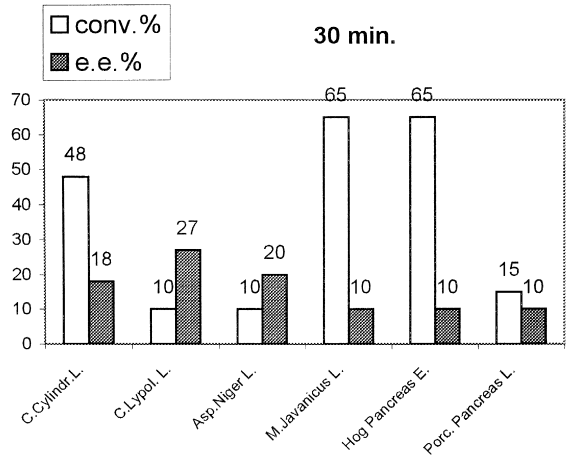



Fig. 1. Reaction course of resolution of substrates **5b** in water with various lipases as catalysts.

the parent compounds of type **4** . From these experiments, we conclude that the bromo derivative will not be considered further for resolution.

Compound **6b** was examined next. 50 mM suspensions of the product in 0.1 M buffer pH 7.5 were shaken in the presence of the enzyme (10 U) at room temperature. Samples were withdrawn every 30 min and the mixtures analysed initially by tlc to follow the degree of hydrolysis. Once determined, the time at which 50% hydrolysis was observed, the products were isolated and the ee measured by HPLC on chiral stationary phase. In aqueous solution, the reaction was followed for 2 h, the results are reported in Fig. 2a and b.

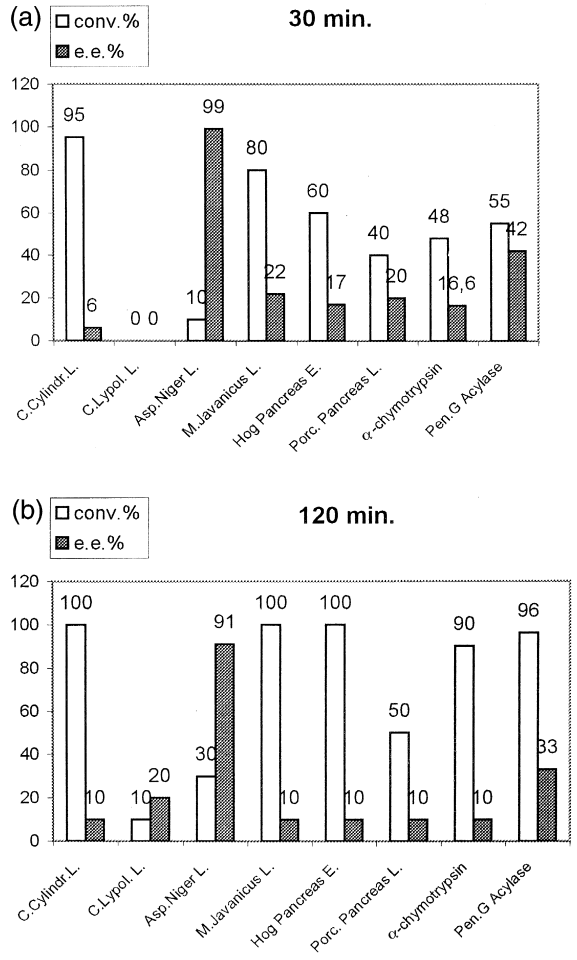



Fig. 2. (a) Reaction course of resolution of substrates **5b** in water with various lipases as catalysts (30 min). (b) Reaction course of resolution of substrates **5b** in water with various lipases as catalysts (120 min).

These results show that lipase from *A. niger* is cooperative in a first screening up to 30% conversion. It is well known that a partial positive result can be often brought to application by reaction engineering and catalyst engineering. We have recently shown that substrate control with hydrophobic absorbing resins can influence both yields and selectivity in whole cell biotransformations [8]. Increased selectivity with increased dilution is believed to be a simple kinetic control in multi-enzymatic systems. Due to the low purity of commercial biocatalysts like the lipase from *A. niger*, we envisaged the possibility of improving the enantioselectivity of the biotransformation by adsorbing the substrate on one of such supports. We found that when the substrate was diluted 1:1 in a styrene–DVB copolymer, the reaction course was sensibly improved and 90% ee was obtained at 50% conversion. This result can be considered as satisfactory at the light of the fact that the obtained material will undergo further synthetic steps with solid intermediates prone to easy purification by crystallisation. From the results observed, the possibility that the improvement is due to the selective adsorption of a specific enzyme present in the mixture, or to some effect of selective activation due to differential immobilization, cannot be completely ruled out. The hydrolysis in water saturated solvents showed the possibility of a change in selectivity, but ee were not satisfactory.[1]

In conclusion, a simple screening among crude commercially available hydrolytic enzymes allows the identification of a catalyst adequate for the preparation of single enantiomers in sufficient enantiomeric purity for practical applications.

## Acknowledgements

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[1] Best results were obtained, among a set of 10 hydrophobic resins tested, with Sepabeads SP207, a synthetic adsorbent resin based on a styrene–DVB copolymer with a very high degree of hydrophobicity (solubility index 10.7).

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