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Chemo-enzymatic synthesis of (*R*)- and (*S*)-3,4-dichlorophenylbutanolide intermediate in the synthesis of sertraline

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Abstract

3,4-Dichlorophenacylchloride was reduced with whole cell biocatalysts to give the (*R*)- or (*S*)-chlorohydrine in high yields and good to high enantiomeric excess. Yields and enantiomeric purity of the (*S*)-enantiomer were increased to 95 and >98%, respectively, using growing cells from *Geotrichum candidum* (CBS 233.76) in the presence of hydrophobic adsorbing resins at 4 g/l. The latter compound was transformed into (*R*)-3,4-dichlorophenylbutanolide, intermediate in the synthesis of (+)-*cis*-1*S*,4*S*-sertraline.

1. Introduction

The synthesis of chiral compounds in enantiomerically pure form has become an unavoidable task in the production of chiral drugs and biologically active materials.[1–3] Biocatalytic methods compete with classical synthetic approaches. Since in many cases the key compound in a synthetic step is an intermediate bearing a secondary alcohol function, the asymmetric reduction of ketones is one of the more useful reactions in the production of chirality. In recent times the discovery of asymmetric reduction and hydrogenation catalysts (homogeneous and heterogeneous)[4,5] has led to successful industrial applications. However, the potential for these catalysts largely depends on the structural features of the substrate and must be evaluated on a case by case basis. On the other hand the use of biocatalysts for the asymmetric reduction of ketones relies on the availability of a suitable enzyme or whole cell biocatalyst. The latter form is usually preferred on an industrial scale in order to avoid the problem of cofactor regeneration.[6] Also in this case, a special catalyst must be developed for each substrate. However, there are a number of easy to grow GRAS (generally recognized as safe) micro-organisms whose substrate

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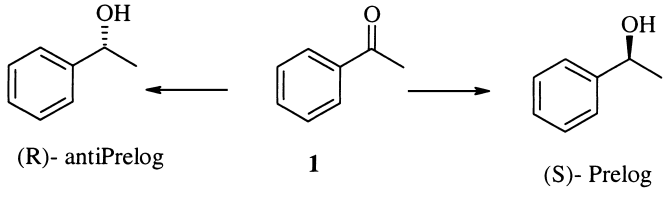
specificity is rather large.[7] Among them a useful and efficient biocatalyst is often found. Moreover, although the prevalent enantiopreference in the reduction gives alcohols with (*R*) absolute configuration, micro-organisms giving alcohols belonging to the (*S*) family can be encountered.[8–11] Benzyl alcohols derived from the reduction of the corresponding aromatic ketones have been widely investigated for the preparation of a large group of α- and β-adrenergic drugs.[6]

Substrates bearing a phenyl ring and an alkyl group on the two sides of a carbonyl group are among the most extensively studied substrates for dehydrogenase enzymes. Acetophenone **1** is an ideal substrate for assaying the stereochemical preference of a reducing biocatalyst.[7,12,13] Due to the large difference in stereochemical requirements of the two groups flanking the carbonyl function, a simple experiment allows a micro-organism to be assigned to the Prelog specificity family or to the opposite anti-Prelog one (Scheme 1). Although many data are available for the reduction of this compound, very few examples are reported where the aromatic ketone bears the required functional groups found in target molecules.[6,14]



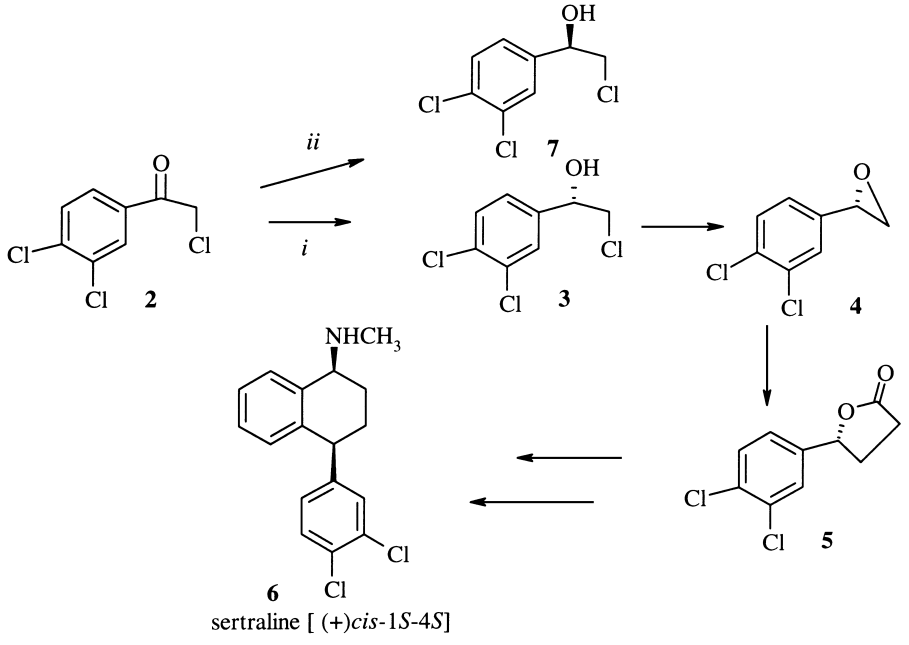
Scheme 1.

We report in this article the biocatalytic reduction of 3,4-dichloro-α-chloroacetophenone to give the corresponding chlorobenzyl alcohols in both absolute configurations. The alcohol of (*S*)-absolute configuration is then transformed into **5**, an intermediate in the synthesis of sertraline.[15]

2. Results and discussion

Thus, 3,4-dichloro-α-chloroacetophenone **2** was assayed as a substrate for a series of micro-organisms from our collection which are known for their high reducing capacity. Assays were performed by adding 50 mg of substrate to the growing cells at 1 g/l concentration. The extent of conversion was monitored by GC of an organic extract, while the enantiomeric excess of the product was determined by GC on chiral stationary phase. Several micro-organisms were identified which give as a product the chlorohydrin with the (*R*) absolute configuration (note the change of descriptor with respect to acetophenone due to priority rules). Baker's yeast, for instance, gives **7** in 95% conversion but only 41% e.e. The same compound can be obtained in 88% yield and >99% e.e. with growing cultures of *Rhodotorula mucillaginosa* (CBS 2378). The absolute configuration has been determined by comparison of the specific rotation with the data of lactone **5** known from the literature.[15] The latter compound was obtained as described in the following. Compound **3** with the absolute configuration required for the conversion outlined in Scheme 2 was obtained by biotransformation with a strain of *Geotrichum candidum* and was isolated in 90% yield and 93% e.e. At 2 g/l the reaction was still complete in 48 h and the enantiomeric excess of the chlorohydrin remained unchanged. At higher substrate concentration the conversion was considerably lower, indicating the presence of product inhibition. In order to compete with asymmetric methods employing hydrogenation catalysts, space-time yields of the transformation are crucial. We have recently shown the beneficial effect of the presence of hydrophobic adsorbing resins in the selectivity and efficiency of whole cell biotransformations.[16–18] The addition of adsorbing resins during the biotransformation lowers the actual concentration in the surrounding cells, improving the selectivity and the product isolation. The presence of Amberlite XAD-1180 at 2:1 weight to weight ratio

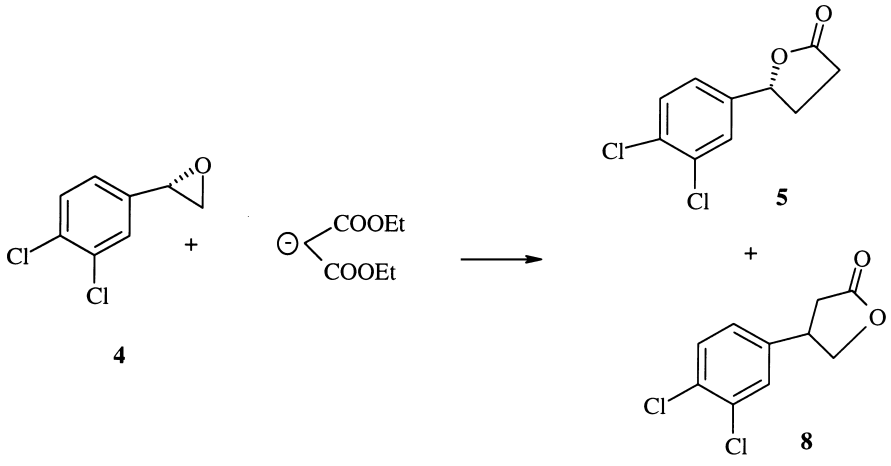
with the substrate allows results in compound **3** to be obtained in 95% yield and with an enantiomeric excess higher than 98% at concentrations of 4 g/l. In this way compound **3** was isolated in gram amounts (see Experimental). Attempts to maximize the substrate concentration have not been made. The product was purified by distillation and quantitatively transformed into the corresponding epoxide without any loss in the enatiomeric excess as established by GC on chiral stationary phase and comparison with a racemic specimen.



Scheme 2. (i) *G. candidum*: 90% yield, 93% ee, 2 g/l (with absorbing resins XAD-1180: 95% yield, >98% ee, 4 g/l); (ii) baker's yeast: 95% yield, 41% ee. *Rhodotorula mucillaginosa*: 88% yield, >99% ee, 1 g/l

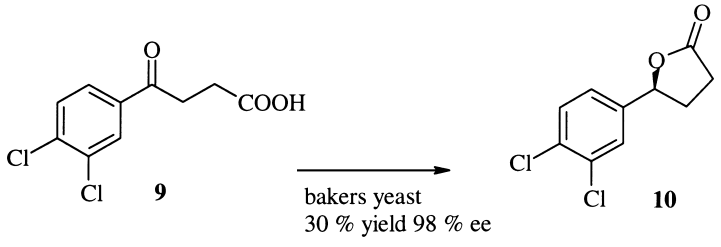
The opening of styrene oxides bearing different substituents on the aromatic ring with nucleophiles has been analysed in the case of *para* substituted compounds. The regioselectivity of the displacement reaction has been correlated with the electronic character of the substituent.[19] While styrene oxide on reaction with malonate anion gives a mixture of the two isomeric products, *p*-OCH_3 and *p*-NO_2, styrene oxides give the product of ring opening in positions 2 and 1, respectively, with high regioselectivity. In our case a 1:1 mixture of the two products **5** and **8** was obtained in an attempted reaction with diethylsodium malonate in absolute ethanol[20] (Scheme 3).

The use of different solvents and different cations in the opening reaction proved that the selectivity is deeply influenced by these factors. Sato et al.[21] have previously shown that non-protic solvents favour the ring opening in the 2-position of the oxirane. Dioxane gave the best results in our case. Thus, the anion was generated in ethanol with sodium ethoxide, the solvent replaced with dioxane and the reaction conducted at reflux. The regioselectivity to give **5** was complete giving the required compound in 70% yields and 95% e.e. Lithium or potassium malonates in EtOH or different solvents such as diethyl ether, MTBE or THF did not give any improvement in the observed regioselectivity which, in these solvents, was only modest. Thus, the combination of a co-operating micro-organism and simple reaction engineering allows the lactone **5** to be obtained in good yield and enantiomeric excess, whose conversion into the antidepressant sertraline is known.[15] In another approach (Scheme 4) compound **9** was directly reduced to the hydroxy acid, which spontaneously cyclized to give the lactone whose configuration was determined as (*S*) from rotation values and GC on chiral column. The isolated yield of the lactone was



Scheme 3.

not improved in this case by the use of absorbing resins, nor could the (*R*)-enantiomer be obtained from **9** by a simple micro-organism screening.



Scheme 4.

The results reported in this article show the versatility of whole cell biocatalysts in the preparation of enantiopure chiral compounds.

3. Experimental

3.1. Analytical

GC chiral analysis were performed on a DANI 8610 with an FID detector, fitted with a glass capillary column. Megadex DetTBuSiβ-cdx (Mega, Legnano Italy), 25 m×0.25 mm i.d., film thickness 0.25 μm for compounds **3**, **4** and **7**; Chirasil-dex CB (Chrompack) 25 m×0.25 mm i.d., film thickness 0.25 μm for compounds **5** and **10**. Rotations were recorded with a Propol automatic digital polarimeter. ^{1}H NMR were recorded on a Varian EMX 250 MHz with TMS as internal standard; all spectra were recorded in $CDCl_3$ unless otherwise indicated.

3.2. Adsorption of the substrates onto the resin

The crude commercial resin XAD-1180 was subsequently washed with deionized water and acetone (3 ml for 1 ml of resin). The substrate was dissolved in acetone and the resin, once dried, added to the solution [i.e. substrate (1 g), acetone (10 ml) and dry resin (2 g)]. The mixture was shaken for 10 min,

avoiding the use of a magnetic bar which could damage the resin, and then the acetone was evaporated at reduced pressure. The solid so obtained was poured directly into the fermentation flask.

3.3. 2-Chloro-1-(3,4-dichlorophenyl)ethanone **2**

Prepared as reported in the literature[22] (1,2-dichlorobenzene, chloroacetylchloride, $AlCl_3$, CS_2, 50°C, 24 h). Anal calcd for $C_8H_5Cl_3O$: C, 43.00; H, 2.26; Cl, 47.59. Found: C, 43.08; H, 2.31; Cl, 47.52.

3.4. 2-Chloro-(1S)-(3,4-dichlorophenyl)ethanol **3**

To a culture of *G. candidum* in MPGB medium (50 ml) [D-glucose (20 g/l), peptone (5 g/l) malt (20 g/l)], in a 300 ml Erlenmeyer flask, **2** (200 mg, 0.89 mmol) adsorbed onto XAD-1180 (400 mg) was added. A total of 10 flasks were used for the experiment. The culture was left for 48 h at 28°C on a linear shaker at 120 movements/min. The crude fermentation medium (500 ml) was filtered over a sintered glass funnel (porosity 0). The resin residue was washed with water (150 ml) and was then extracted with ethyl acetate (3×150 ml). The organic solvent, once dried and evaporated, left a brown oil which was distilled in a Kugelrhor apparatus (100°C, 0.1 mmHg) so as to obtain pure **3**, 1.9 g (8.4 mmol, 95% yield), $[\alpha]_D$=+32.5 (*c* 1, $CHCl_3$), ee 98.5% (GC, DetTBuSiβ-cdx); ^{1}H NMR: δ 1.87 (1H, OH, broad), 3.58 (1H, CH_2, dd), 3.72 (1H, CH_2, dd), 4.87 (1H, CH, dd) and 7.20–7.52 (3H, Ph, m). Anal calcd for $C_8H_7Cl_3O$: C, 42.61; H, 3.13; Cl, 47.17. Found: C, 42.68; H, 3.09; Cl, 47.20.

3.5. 2-Chloro-(1R)-(3,4-dichlorophenyl)ethanol **7**

Microbiological reduction similar to that for the preparation of **3**. Growing cultures of *R. mucillaginosa* instead of *G. candidum* were used in MPGB medium. The transformation was conducted at 1 g/l initial concentration without the use of adsorbing resins. Conversion was complete at 48 h. Compound **7** was recovered from the culture medium by extracting the aqueous phase with ethyl acetate. Yield of distilled **7** was 88%, $[\alpha]_D$=−32.7 (*c* 1, $CHCl_3$), ee >99% (GC, DetTBuSiβ-cdx); ^{1}H NMR: δ 1.87 (1H, OH, broad), 3.59 (1H, CH_2, dd), 3.72 (1H, CH_2, dd), 4.86 (1H, CH, dd) and 7.20–7.52 (3H, Ph, m). Anal calcd for $C_8H_7Cl_3O$: C, 42.61; H, 3.13; Cl, 47.17. Found: C, 42.65; H, 3.11; Cl, 47.19.

3.6. (2S)-(3,4-Dichlorophenyl)oxirane **4**

Compound **3** (2 g, 8.8 mmol) was dissolved in ethanol (15 ml) and NaOH (0.75 g, 18 mmol), dissolved in water (10 ml), was added in one portion. The mixture was stirred at 25°C for 3 h and the ethanol evaporated at reduced pressure. The aqueous phase was extracted with diethyl ether (3×15 ml); the organic extract, once dried and evaporated under vacuum, left a clear oil. The oily residue was distilled in a Kugelrhor apparatus (100°C, 1 mmHg) so as to obtain pure **4**, 1.5 g (8 mmol, 90% yield), $[\alpha]_D$=+15.6 (*c* 1, $CHCl_3$), ee 98.6% (GC, DetTBuSiβ-cdx); ^{1}H NMR: δ 2.72 (1H, CH_2, dd), 3.14 (1H, CH_2, dd), 3.81 (1H, CH, dd), 7.11 (1H, Ph, m) and 7.48 (2H, Ph, m). Anal calcd for $C_8H_6Cl_2O$: C, 50.83; H, 3.20; Cl, 37.51. Found: C, 50.88; H, 3.21; Cl, 37.52.

3.7. (5R)-(3,4-Dichlorophenyl)dihydrofuran-2-one **5**

Sodium (0.18 g, 7.8 mmol) was dissolved in anhydrous EtOH (10 ml) and diethyl malonate (1.2, 7.8 mmol) was added dropwise. The mixture was heated at reflux for 30 min, keeping the reaction vessel

under nitrogen. The ethanol was distilled off in a stream of nitrogen and the solid residue was re-dissolved in dry dioxane (15 ml). The mixture was heated at reflux and the epoxide **4** (1.5 g, 7.9 mmol), dissolved in dry dioxane (5 ml), was added in 10 min. The reaction was refluxed for 3 h. NaOH (0.15 g, 3.9 mmol) in water (10 ml) was then added and dioxane was distilled off. The residue aqueous solution was acidified with conc. HCl (1.1 ml) and extracted with CH_2Cl_2 (2×20 ml). No heating was required to induce decarboxylation. The crude extract was purified by SiO_2 chromatography using as eluent a mixture of *n*-hexane and ethyl acetate so as to obtain pure **5** (1.2 g, 5.2 mmol, 65% yield), mp 67°C, $[\alpha]_D$=+11.5 (*c* 1, MeOH) and +18.6 (*c* 1, $CHCl_3$), ee 95% (GC, Chirasil-dex CB); ^{1}H NMR: δ 2.06–2.27 (1H, CH_2, m), 2.60–2.70 (3H, $2CH_2$, m), 5.45 (1H, CH, m), 7.17 (1H, Ph, m) and 7.41–7.50 (2H, Ph, m). Anal calcd for $C_{10}H_8Cl_2O_2$: C, 51.98; H, 3.49; Cl, 30.68. Found: C, 51.95; H, 3.42; Cl, 30.62.

*3.8. (5S)-(3,4-Dichlorophenyl)dihydrofuran-2-one **10***

In a 5 l beaker containing tap water (2 l) at 37°C D-glucose (100 g) was dissolved and fresh baker's yeast (500 g) (Distillerie Italiane, Eridania group) was added portionwise. The mixture was stirred for 30 min. The hydroxy acid **9**[23] (5 g, 20.3 mmol) dissolved in EtOH (50 ml) was added in 15 min to the fermenting yeast and the mixture was stirred at 25°C for 48 h. The reaction medium was filtered over a pad of Celite® and the aqueous phase was extracted with ethyl acetate (3×250 ml). The collected organic phases, once dried and evaporated, gave a thick oil which was purified on SiO_2 chromatography using as eluent a mixture of *n*-hexane and ethyl acetate so as to obtain pure **9** (1.4 g, 6.1 mmol, 30% yield), white solid, mp 65°C, $[\alpha]_D$=−12.1 (*c* 1, MeOH), ee 98% (GC, Chirasil-dex CB); ^{1}H NMR: δ 2.08–2.27 (1H, CH_2, m), 2.61–2.73 (3H, $2CH_2$, m), 5.45 (1H, CH, m), 7.16 (1H, Ph, m) and 7.41–7.52 (2H, Ph, m). Anal calcd for $C_{10}H_8Cl_2O_2$: C, 51.98; H, 3.49; Cl, 30.68. Found: C, 51.98; H, 3.47; Cl, 3.70.

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