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Highly stereoselective preparation of (3*R*,4*S*)-3,4-chromanediol by deracemization of (±)-3-hydroxy-4-chromanone by *Trichosporon cutaneum*

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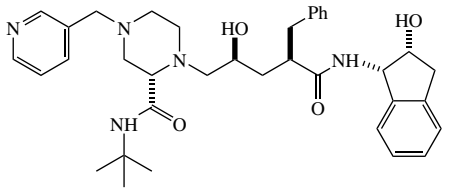
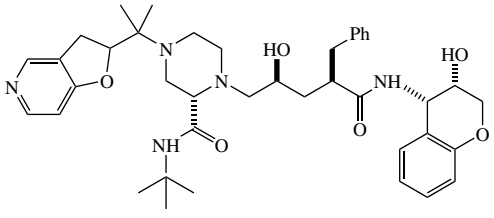
Abstract—Deracemization of (±)-3-hydroxy-4-chromanone **2** through stereoselective bioreduction to the corresponding (3*R*,4*S*)-3,4-chromanediol **3**, with good to excellent enantiomeric excesses (up to 99%), mediated by resting cells of the yeast *Trichosporon cutaneum* CCT 1903, is reported. In addition, 3-hydroxychromone (**7**) was obtained as a secondary product.

1. Introduction

Several HIV protease inhibitors, including Indinavir, have been approved by the US Food and Drug Administration as important therapeutic agents for the treatment of HIV infections and AIDS.[1] Although these drugs represent a major advancement in the treatment of HIV disease, they suffer from first-pass metabolism, some food restrictions and a number of patients develop resistance to multiple protease inhibitors through viral mutations.[2] To overcome these restrictions, there have been extensive investigations searching for Indinavir analogues with improved potency against virus and protease inhibitor resistant HIV strains. Merck Research Laboratories recently demonstrated that substitution of aminochromanol residue of Indinavir by aminoindano afforded a compound with greater potency (Fig. 1).[3]



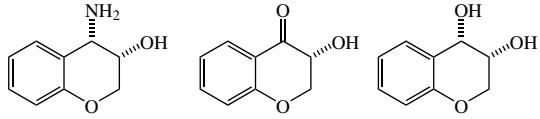
Indinavir



Merck Indinavir analogue

Figure 1.

Hansen et al. synthesized *cis*-4-aminochromanol-3-ol **1** starting from homochiral 3-hydroxy-4-chromanone **2** and they observed that it was not possible to obtain **1** starting from chromene via an asymmetric epoxidation followed by the Ritter reaction.[4] In this context, *cis*-(3*R*,4*S*)-3,4-chromanediol **3** is an important precursor



1 **2** **3**

Figure 2.

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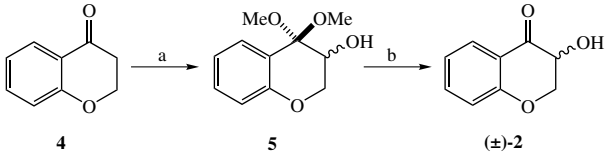
in the synthesis of (3*S*,4*S*)-4-amino-3-chromanol **1** (Fig. 2).

Diol **3** was thought to be converted to aminochromanol **1** through a Ritter-type reaction.[5] On the other hand, this and the related amino alcohols have been used successfully in a conformational toolbox of oxazoline ligands and benzopyrane-type potassium-channel activators.[6] Hitherto, to the best of our knowledge, only two biocatalytic routes addressing **3** have been achieved; Boyd et al.[7] reported the one-step synthesis of **3** (10% yield, >98% ee) by procaryotic (bacterial) dioxygenase-catalyzed dihydroxylation of chromene, whilst Holland et al.[8] found that the fungus *Mortierella isabellina* catalyzes the biotransformation of chromenes to give both (3*R*,4*S*)-*cis* and (3*R*,4*R*)-*trans*-diols **3** in 78% ee and 2% and 4% yield, respectively. However, the drawbacks of these methods relate to the poor yields of the desired diol.

Recently, we reported the diastereo- and enantioselective preparation of enantiopure (1*S*,2*R*)-1,2-indanediol (75% yield, >99% ee) by the asymmetric reduction of 1,2-indane-1,2-dione mediated by cells of the non-conventional yeast *Trichosporon cutaneum* CCT 1903.[9] Following our continuing interest in obtaining synthetically useful enantiomerically pure compounds through whole yeast cells-mediated biocatalysis, we herein report the preparation of diol **3** in high enantiomeric excess by deracemization (kinetic resolution) of racemic 3-hydroxy-4-chromanone **2** through the stereoselective reduction mediated by fresh resting cells of *T. cutaneum* CCT 1903.

2. Results and discussion

Racemic **2** was prepared through hypervalent iodine oxidation as previously reported by Moriarty.[10] As such, commercially available 4-chromanone **4** was converted to 3-hydroxy-4-chromanone dimethylketal **5** in 84% yield with methanolic KOH and iodobenzenediacetate. Hydrolysis of ketal **5** was performed by aqueous HCl (3 mol L^{-1}) in ethanol (45 min) to give racemic benzoin **2** in 96% yield (Scheme 1).



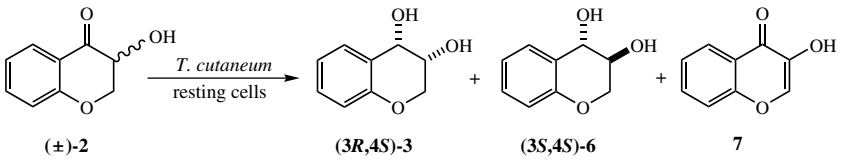
Scheme 1. Reagents and conditions: (a) MeOH/KOH, $C_6H_5I(OAc)_2$, 0°C–rt, overnight, 84%; (b) HCl 3 mol/L, EtOH, rt, 45 min, 96%.

The biotransformation of (±)-**2** by fresh resting cells[11] of *T. cutaneum* CCT 1903[12] was carried out in phosphate buffers at different pH values and the influence of added glucose evaluated. Typically, racemic **2** was converted into a mixture of products after 48 h of incubation (Scheme 2). In all experiments, the major product was identified as *cis*-(3*R*,4*S*)-diol **3** with enantiomeric excesses ranging from 92% to 99% (Table 1).[13,14] The minor product proved to be the *trans*-(3*S*,4*S*)-diol **6**,[15] recovered with high enantiomeric excess >98% and the other secondary compound was identified as 3-hydroxychromone **7**.[16]

The results depicted in Table 1 show that, in the experiments at higher pH values, an enhancement in ee of (3*R*,4*S*)-diol **3** (both in the absence and presence of glucose) was achieved (entries 3, 6 and 8). Clearly there is a compromise between the pH of the medium, the yield, and the enantiomeric excess of diol **3**: the higher the pH the higher the ee and the lower the isolated yield (Table 1). The results presented in Table 1 show that experiments performed at different pH, with or without the glucose, had similar yields and have not shown a significant variation in ee of enantiomers of *cis*-diol **3**. The decrease of the yields of the diols at pH $\geqslant$ 7 is a consequence of oxidation of **2**, since we found that alkaline media increases the yield of chromone **7**.

To evaluate reaction reproducibility, experiments using phosphate buffers at pH 8 were performed on a larger scale (0.5 g of substrate) in the absence and presence of glucose. In the experiment performed in the absence of glucose, the ee of *cis*-diol **3** shows a significant decrease during the course of the reaction, mainly after 20 h. On the other hand, the experiment performed in the presence of glucose showed only a slight decrease in the enantioselectivity. After 46 h (total consumption of substrate), the yeast cells were removed by centrifugation from the reaction without glucose, while diol **3** was recovered from the aqueous phase by extraction with ethyl acetate. Purification by flash column chromatography gave **3** in 52% yield with an ee 73%. In the experiment performed with glucose, diol **3** was obtained in 45% yield and 96% ee (Graph 1). The 3-hydroxychromone **7** and *trans*-diol **6** also were obtained in 21% and 3% yields in the absence of glucose and in 24% and 2% yields with glucose present.

In a blank experiment (no yeast cells) at pH 10 (28 °C), the complete consumption of **2** to quantitatively yield chromone **7** after 22 h was observed. Therefore, air must play a crucial role in the formation of **7**, although the contribution of the enzymatic oxidation to some degree is not ignored as discussed below. As we have found in a

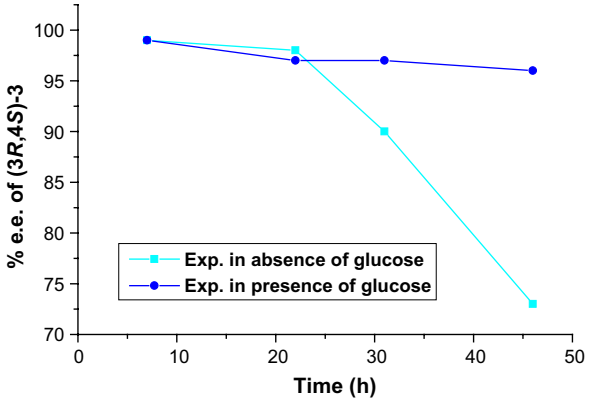


Scheme 2.

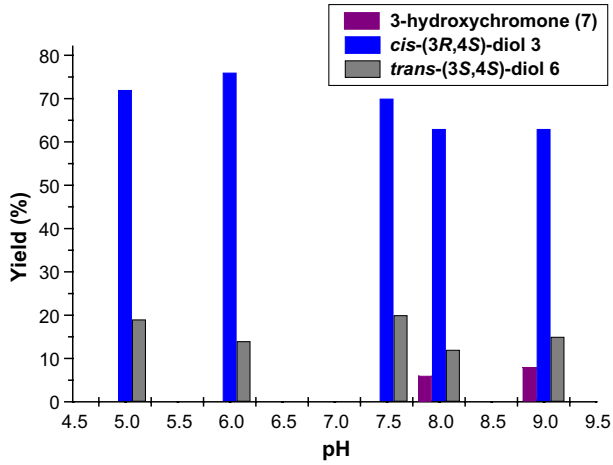
Table 1. Influence of the pH and glucose on the biotransformation of (±)-**2** at 28 °C

Entry	pH[b]	Time (h)	Yield (%)[a]			ee (%)[c] (3*R*,4*S*)-diol 3
			Chromone 7	*cis*-Diol 3	*trans*-Diol 6[g]	
1	5[d,f]	48	2	58	15	92
2	5[e,f]	48	5	54	4	93
3	7[d]	48	21	56	—	97
4	7[e]	48	20	56	3	95
5	8[d]	48	25	45	—	99
6	8[e]	48	26	45	—	98
7	9[d]	48	18	43	10	97
8	9[e]	48	15	41	—	99
9	10[d]	48	32	40	—	99
10	10[e]	48	31	38	—	99

[a] Isolated yield.
[b] Experiments carried out in a 0.05 g scale of (±)-**2** without buffer pH correction during the reaction.
[c] Absolute configurations of **3** and **6** were determined by comparison of experimental specific rotation and that found in the literature.[7]
[d] Reaction performed in absence of glucose.
[e] Reaction performed in presence of glucose (1.0 g).
[f] In distilled water, that is, initial pH after adding cells to distilled water.
[g] >98% ee.



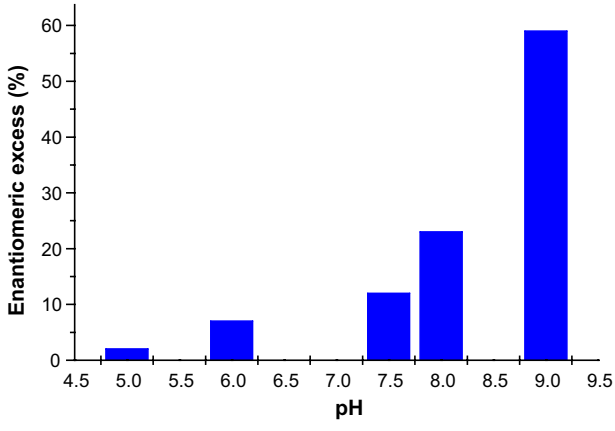
Graph 1. % ee for (3*R*,4*S*)-**3** as a function of the time after 46 h of incubation at 28 °C with resting cells of *T. cutaneum*, pH = 8.



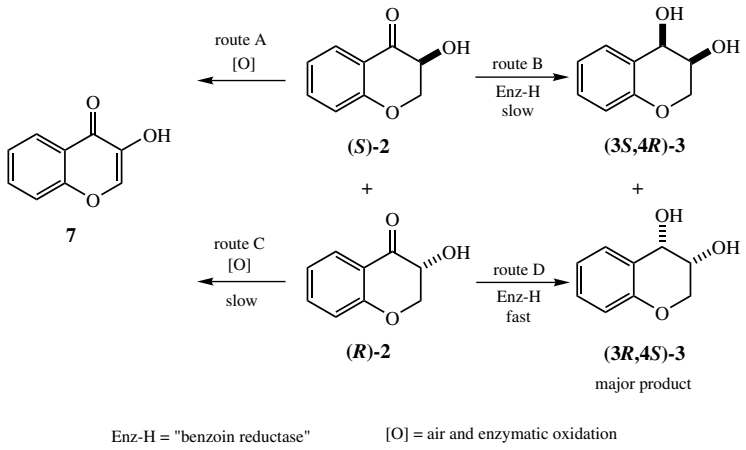
Graph 2. Isolated yield for diols **3** and **6** and chromone **7** as a function of the pH of the reaction medium after 90 h of incubation at 28 °C with resting cells of *T. cutaneum* under anaerobic conditions.

parallel experiment that chromone **7** is not reduced at all by *T. cutaneum*, it seems that the variation of ee (for product **3**) should be related to two individually connected enzymatic events: (1) reduction of (*R*)-**2** is far faster than the reduction of (*S*)-**2** by a 'benzoin reductase', present in the kinetic resolution process; (2) in addition to the oxidation of both (*R*)-**2** and (*S*)-**2** by atmospheric O_2, a faster enzymatic oxidation of (*S*)-**2** should be evoked, that is, a kinetic resolution process, since experiments in the same pH performed in the presence and the absence of cells showed that the (*S*)-**2** is oxidized faster in the presence of cells than in their absence.

Consequently, shifting from an acidic to an alkaline media, caused a drop in the yield of **3** but an increase in the yield of chromone **7**. As the latter compound is the oxidation product of **2**, we hypothesized that it could be the result of competing oxidative and reductive enzymes acting over the same substrate as a function of the medium's pH. As such, we reasoned that a more acidic media favored the reductive enzymes whilst more basic ones favored the oxidative enzymes. New experiments under anaerobic conditions to investigate the



Graph 3. Enantiomeric excess of diol **3** as a function of the pH of the reaction medium after 90 h of incubation at 28 °C with resting cells of *T. cutaneum* under anaerobic conditions.



Scheme 3.

O_2-dependent oxidation of chromanone **2** were performed. The results, shown in Graph 2, reveal that formation of the oxidation product **7** was not observed below pH 8. Unfortunately, these reduction processes in anaerobic conditions took place with significant racemization of diol **3** (Graph 3). At pH 5, diol **3** was isolated in almost racemic form and compound **7** was absent. When the yield of the oxidation product **7** increased, an increase in the enantioselectivity of diol **3** was also observed (Graphs 2 and 3). This odd observation is explained as follows: under anaerobic conditions, the oxidation of enantiomer (*S*)-**2** to chromone **7** is quite slow in comparison with the aerobic conditions. Hence (*S*)-**2** is slowly reduced to antipode (3*S*,4*R*)-**3**, ultimately decreasing drastically the ee of (3*R*,4*S*)-**3**.

Overall, Scheme 3 shows that under aerobic conditions the fast oxidation of (*S*)-**2** to **7** and the rapid reduction of (*R*)-**2** prevail (route A and D). On the other hand, under anaerobic conditions, route D prevails over route C and route B is predominant over route A with (3*R*,4*S*)-**3** obtained in low ee.

3. Conclusion

In conclusion, this is the first report addressing the deracemization of (±)-3-hydroxy-4-chromanone **2** to furnish (3*R*,4*S*)-3,4-chromanediol **3**. In addition, the yield (45%) obtained for diol **3** (99% ee) in phosphate buffer at pH 8, although modest, is the best yield reported so far.

Acknowledgements

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11. Typical procedure: To a slurry of 3 g (wet weight) of *Trichosporon cutaneum* CCT 1903 and glucose (1 g) in different phosphate buffers (50 mL), a solution of (±)-**2** (50 mg) in ethanol (0.5 mL) was added. Experiments were also performed in different phosphate buffers (50 mL) in the absence of glucose. The resulting suspensions were stirred on an orbital shaker (150 rpm) at 28 °C until complete consumption of **2**. After centrifugation, the supernatants were thoroughly extracted with ethyl acetate. Purification of the crude product was achieved by flash column chromatography on silica gel, using hexane/ethyl acetate (2:1 and 1:1) as eluent.
12. This strain is stored at 'André Tosello' Research Foundation (Brazil). Synonym *Trichosporon beigelli* (Kuechen-

meister & Rabenhorst) Vuillemin. It was cultivated in 2% SDB (Sabouraud dextrose broth, 1 L) for 2–3 days incubation at 28 °C on an orbital shaker (150 rpm) before use. The cells were harvested by centrifugation (5000 rpm).

13. The absolute configurations were assigned by comparison of the sign of the specific rotation with that reported in the literature.[7] Data for (3*R*,4*S*)-**3**: white crystals, mp 168–171 °C (lit.[7] 159–161 °C); $[\alpha]_D^{20} = +77.5$ (*c* 0.87, THF) {lit.[7] $[\alpha]_D^{20} = +63$ (*c* 0.77, THF) for >98% ee}; ^{1}H NMR (500 MHz, $CHCl_3$) δ 2.48 (m, 2H, OH); 4.14 (m, 3H); 4.77 (m, 1H); 6.85 (dd, $J = 9.0$ and 1.0 Hz, 1H); 6.98 (td, $J = 7.5$ and 1.0 Hz, 1H); 7.23 (m, 1H); 7.41 (dd, $J = 7.75$ and 1.25 Hz, 1H); ^{13}C NMR (125 MHz, $CHCl_3$) δ 65.8; 65.9; 66.0; 116.6; 121.4; 122.6; 129.8; 129.9; 153.6; IR (KBr) 3260; 1610; 1584; 1488; 1457; 1235; 1039; 761 cm^{-1}; MS *m*/*z* 166 ($M^{+\cdot}$, 33%), 148 (3%), 131 (5%), 120 (84%), 121 (100%), 104 (14%), 91 (11%), 77 (16%), 65 (12%), 51 (8%), 43 (5%). HRMS *m*/*z* found: 166.06089; Calcd for $C_9H_{10}O_3$: 166.06299.
14. The ee of diol **3** was determined by GC–MS (Shimadzu QP-5000) analysis through a Chirasil-dex capillary chiral column (30 m × 0.25 mm × 0.25 μm), after isolation by flash column chromatography (vide supra Ref. 11) and conversion to the corresponding acetonide derivative.
15. Data for (3*S*,4*S*)-**6**: colorless oil, $[\alpha]_D^{20} = +23$ (*c* 0.3, THF) {lit.[7] data for enantiomer (3*R*,4*R*)-**6** with >98% ee $[\alpha]_D^{20} = -22$ (*c* 0.49, THF)}; ^{1}H NMR (300 MHz, $CHCl_3$) δ 2.23 (m, 2H, OH); 3.99 (m, 1H); 4.13 (ddd, $J = 11.5$; 5.3 and 1.1 Hz, 1H); 4.27 (dd, $J = 11.4$ and 2.4 Hz, 1H); 4.59 (m, 1H); 6.87 (d, $J = 8.7$ Hz, 1H); 6.98 (td, $J = 7.5$ and 1.1 Hz, 1H); 7.23 (m, 1H); 7.36 (dd, $J = 7.35$ and 1.65 Hz, 1H); IR (KBr) 3449; 3284; 3160; 1487; 1042; 749 cm^{-1}; MS *m*/*z* 166 ($M^{+\cdot}$, 31%), 148 (1%), 131 (3%), 120 (86%), 121 (100%), 104 (14%), 91 (9%), 77 (14%), 65 (11%), 51 (8%).
16. The spectroscopic data of this compound were identical to those reported previously: Constantino, M. G.; Lacerda, V.; da Silva, G. V. J. *J. Heterocycl. Chem.* **2003**, *40*, 369.