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Tetrahedron: *Asymmetry* 14 (2003) 2327–2330

TETRAHEDRON: *ASYMMETRY*

Homochiral (1*S*,2*R*)-1,2-indandiol from asymmetric reduction of 1,2-indanedione by resting cells of the yeast *Trichosporon cutaneum*

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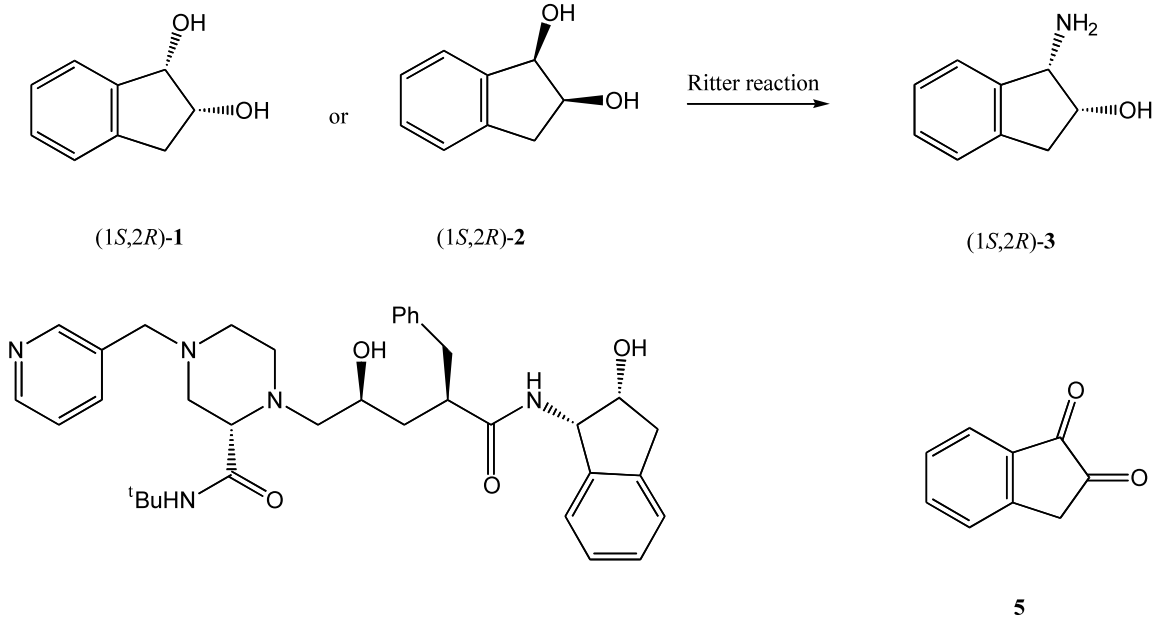
Received 3 December 2002; accepted 3 June 2003

Abstract—A concise highly diastereo- and enantioselective preparation of homochiral (1*S*,2*R*)-1,2-indandiol **1** (75% yield, >99% e.e.) by asymmetric reduction of 1,2-indanedione **5** mediated by fresh resting cells of *Trichosporon cutaneum* CCT 1903 is reported.

(1*S*,2*R*)-1,2-Indandiol **1** is a well established precursor to (1*S*,2*R*)-1-amino-2-indanol **3**, a key raw material in the synthesis of the leading HIV-1 protease inhibitor oligopeptide mimic Indinavir **4** (Scheme 1). Hitherto, only a few biocatalytic routes to **1** have been achieved, e.g. (i) microbial asymmetric dioxygenation of indene[1] and (ii) non-conventional yeast-mediated asymmetric reduction of 1,2-indanedione[2] **5**. Aminoalcohol **3** is also a unique chiral building block in asymmetric organic synthesis, both for chiral auxiliaries and chiral ligands.[3,4] In turn, either diol **1** or **2** can be easily converted to **3** through a Ritter reaction.[5]



Scheme 1.

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doi:10.1016/S0957-4166(03)00487-7

Asymmetric bioreduction of diketone **5** presents an alternative route to **1** with only one report addressing this goal having been released. Chartrain et al.[2] found that growing cells of *Trichosporon cutaneum* MY 1506 can reduce **5** in a 16 g scale to afford a mixture of **1** (52% yield, 99% e.e.) and **2** (5% yield, 26% e.e.) in ca. 8 days.

Recently, we have reported that the regio- and enantioselective reduction of a toxic acyclic enone by the non-conventional yeast *Pichia stipitis* can be tuned by the use of Amberlite XAD-7.[6] Herein we report another non-conventional yeast, *Trichosporon cutaneum* CCT 1903, which is able to reduce diketone **5** to diol **1** efficiently and that the stereochemical outcome of the reaction depends on the physiological status of the whole yeast cells (resting or growing).

In a screening of non-conventional yeasts[7] to reduce α-diketones we found that a strain of *Trichosporon cutaneum* (de Beurm Gougerot & Vaucher) Ota, namely CCT 1903,[8] was equally able to convert diketone **5** to diol **1**, with transient accumulation of benzoin **6** (Scheme 2).

We found that when the reduction of **5** (450 mg L^{-1}) was performed by growing cells[9] of *Trichosporon cutaneum* which have been cultivated for 2–3 days in SDB 2% (Sabouraud dextrose broth) under controlled conditions, full conversion was attained in 96 h and a mixture of *cis*- and *trans*-indandiols (9:1 ratio, respectively) was obtained with 65% overall yield and 94 and 16% e.e., respectively.[10]

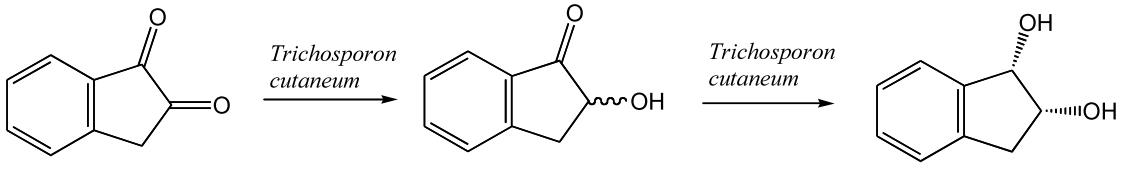
Surprisingly, only the enantiomerically pure (>99% e.e.) *cis*-diol **1** was furnished when the reduction of **5** (750 mg L^{-1}) was carried out for 77 h (full conversion) by fresh resting cells[11] of the same yeast harvested by centrifugation after 2–3 days cultivation in SDB 2% (Scheme 3). No trace of the *trans*-diol **2** was detected by GC/MS analysis.[10] Recovery of the product from the aqueous phase by extraction with ethyl acetate followed by column chromatography furnished pure **1** (70% yield).[12,13]

It is well known that changes in the cell environment (culture medium, pH, temperature, etc.) affect the complex fine tuned cell machinery as the microorganism fits to the new conditions for survival.[15] As such we believe that when the reduction of **5** is mediated by resting cells of the yeast the enzymes involved in the synthesis of (1*S*,2*R*)-**1** remain highly active whereas those with opposite selectivity are mostly inhibited.

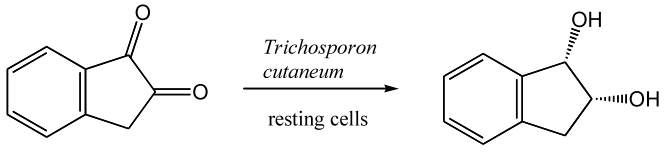
The reaction can be scaled up to reduce 1 g of **5** (1 g L^{-1}) with no changes in the enantiomeric excess and with 75% isolated product yield. Under these conditions, full conversion of **5** was attained in 80 h although a negligible amount (17 mg, <2% yield) of almost racemic *trans*-indandiol was detected by CG/MS analysis through comparison with an authentic sample.

Furthermore our experimental findings reveal that reduction of **5** by resting cells of *Trichosporon cutaneum* is not a simple two step process, i.e. regio- and enantioselective reduction of **5** to form (*R*)-**6** followed by a stereoselective reduction to furnish diol **1**. In fact, monitoring of the reaction by GC/MS analysis with a chiral column disclosed that benzoin **6** was accumulated in racemic form and after a period of many hours (20 h in a 1 g scale reaction) only enantiomer (*R*)-**6** was slowly converted to diol **1** (Scheme 4).

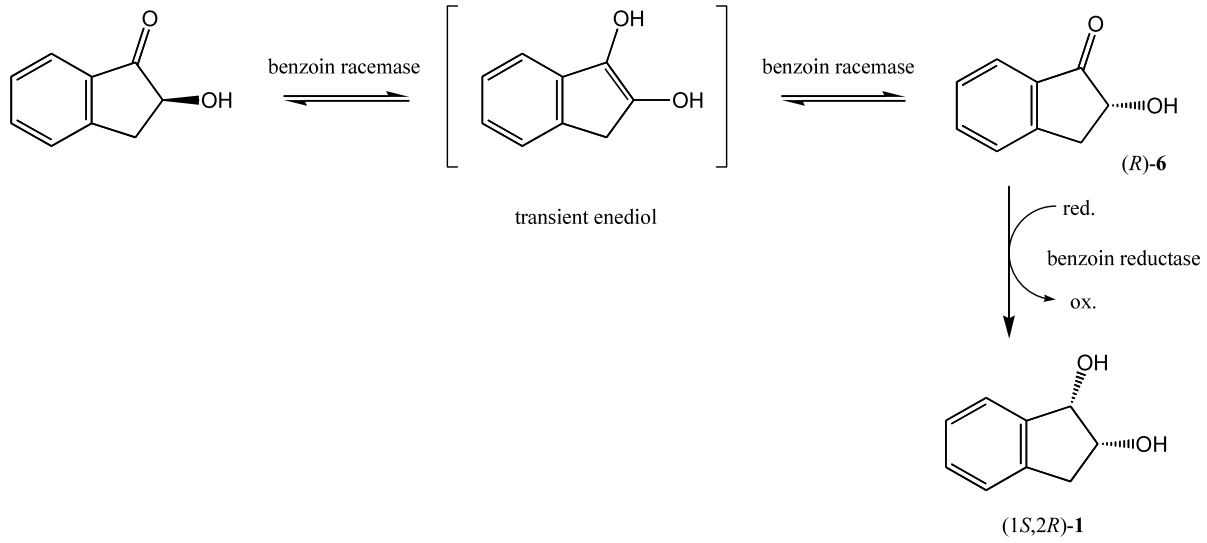
As the faster reacting enantiomer was depleted during the stereoselective reduction to form **1** the equilibrium of (*R*)-**6**/(*S*)-**6** was constantly re-adjusted. Thus, in a concurrent step, the almost unreactive enantiomer (*S*)-**6** was racemized to (*R*)-**6**. The final result was the transformation of a transient racemate to a single stereoisomeric product in high yield.[16] As such, the intervention



Scheme 2.



Scheme 3.



Scheme 4.

of a third enzyme in the overall reaction, a racemase, is envoked (Scheme 4).[17]

It is noteworthy that no special care was needed to perform the reaction with the resting cells of the yeast. Indeed, neither buffer nor pH control was necessary to ensure a good performance of the yeast (the reaction was performed in distilled water), which makes this process suitable for batch scale asymmetric preparation of the title diol. A study for a suitable scale up of the reaction is in progress.

Acknowledgements

The authors are indebted to FAPESP and CNPq for financial support.

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8. This strain is stored at 'André Tosello' Research Foundation (Brazil). Synonym *Trichosporon beigelli* (Kuechenmeister & Rabenhorst) Vuillemin. It was cultivated in SDB 2% (Sabouraud dextrose broth, 1 L) for 2–3 days incubation at 28°C on an orbital shaker (150 rpm) before use. Alternatively, the cells were harvested by centrifugation (5000 rpm).
9. **Typical procedure**: To a slurry of growing *Trichosporon cutaneum* CCT 1903 (250 mL, vide supra Ref. 8), a solution of **5** (150 mg) in ethanol (1 mL) was added. The resulting suspension was stirred on an orbital shaker (150 rpm) at 28°C until total consumption of **5**. After centrifugation, the supernatant was throughly extracted with ethyl acetate. Purification was achieved by flash column chromatography.
10. Enantiomeric excesses were determined by GC/MS analysis (Shimadzu QP-5000) through a Chirasil-Dex fused silica capillary chiral column (30 m×0.25 mm×0.25 μm) with Helium as the carrier gas (1.3 mL min^{-1}). Temperature program: injector 230°C; detector 250°C; oven T_i = 160°C (13 min), rate 20°C min^{-1}, T_f = 190°C (1 min). Retention time (min): (*S*)-**6** (6.1), (*R*)-**6** (6.3), **5** (7.8), (1*R*,2*S*)-**1** (11.7), (1*S*,2*R*)-**1** (12.0), *trans*-diol **2** (14.5 and 14.6).
11. **Typical procedure**: To a slurry of 6 g (wet weight) of *Trichosporon cutaneum* (vide supra Ref. 8) and glucose (4 g) in distilled water (200 mL), a solution of **5** (150 mg) in ethanol (1 mL) was added. The resulting suspension was stirred on an orbital shaker (150 rpm) at 28°C until total consumption of **5**. After centrifugation, the supernatant was thoroughly extracted with ethyl acetate. Purification was achieved by flash column chromatography (hexane/ethyl acetate 1:2 v/v). Yield of (1*S*,2*R*)-**1**: 106.5 mg (70%).
12. Owing to the relative solubility of the diol **1** in the aqueous phase, care must be taken in the extraction step to ensure a good yield (efficient recovery).
13. **Data for (1*S*,2*R*)-1**: White crystals, mp 98–99°C;[14] $[\alpha]_D^{20}$ = −38.8 (*c* 1, $CHCl_3$);[14] ^1H NMR (500 MHz, DMSO-d_6) δ 2.76 (dd, 1H, *J* = 16, 4 Hz), 2.92 (dd, 1H, *J* = 16, 6 Hz), 4.24–4.27 (m, 1H), 4.58 (d, 1H, *J* = 5 Hz), 4.78 (dd, 1H,

$J=7$, 5 Hz), 4.99 (d, 1H, $J=7$ Hz), 7.17–7.20 (m, 3H), 7.30 (m, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 38.3, 72.9, 75.1, 124.8, 124.8, 126.3, 127.6, 140.6, 144.0; IR (KBr) 3523, 3440, 3299, 3155, 2952, 2924, 1459 1322, 1188, 1156, 987, 736, 635 cm^{-1}; MS m/z 150 ($M^{+\bullet}$, 18%), 132 (43%), 131 (33%), 115 (16%), 107 (62%), 104 (100%), 91 (45%), 77 (58%), 65 (28%), 51 (59%), 43 (15%). HRMS m/z found: 150.0679. Calcd for $C_9H_{10}O_2$: 150.0681.

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