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Microbial reduction of cyclohexanones

Roseli Maria De Conti [a,*], André L.M. Porto [a], J. Augusto [a], R. Rodrigues [a], Paulo J.S. Moran [a], Gilson P. Manfio [b], Anita J. Marsaioli [a]

[a] *Instituto de Química, Universidade Estadual de Campinas, Cidade Universitária Zeferino Vaz-Barão Geraldo, 13083-970 Campinas SP, Brazil*

[b] *Fundação Tropical de Pesquisas e Tecnologia André Tosello, 13087-010 Campinas SP, Brazil*

Abstract

A Brazilian strain of the bacteria *Serratia rubidaea* CCT 5742 quantitatively reduced 4-*tert*-butylcyclohexanone and 4-methylcyclohexanone to the less thermodynamically stable diastereoisomeric alcohols *cis*-4-*tert*-butylcyclohexanol and *cis*-4-methylcyclohexanol with a diastereoisomeric excesses (de) of 96% and 44%, respectively. 2-Methylcyclohexanone was also totally reduced to the corresponding alcohols affording the *trans*-(+)-(1*S*, 2*S*)-2-methylcyclohexanol with 78% of de and an enantiomeric excess (ee) of 80%. The *cis*-(+)-(1*S*, 2*R*)-2-methylcyclohexanol was obtained in 98% ee.

Keywords: Diastereo- and enantioselective reductions; Cyclohexanones; *Serratia rubidaea*

1. Introduction

Enzyme-based catalysis is now one of the outstanding synthetic methods to produce enantiopure intermediates, and microorganisms are the treasure tools to these processes allowing the access to both isolated enzymes and whole cell biotransformations [1].

Stereoselective reduction is an ever expanding field specially related to the conversion of cyclic ketones to less thermodynamically stable alcohols [2–5], a niche for synthetic methodology that is still lacking acceptable methods [5].

Reports are particularly scarce on the selective reduction of alkyl cyclohexanones via biocatalysis, notwithstanding the ubiquity of substituted cyclohexanol moieties among natural products [6–10].

Focusing on the reduction of ketones and α,β-unsaturated ketones, screening procedures were carried out with various microorganisms, which were isolated from specific environments like the Brazilian Atlantic forest and spoiled food [11]. Among the six strains of microbes examined, *Citrobacter freundii* (CCT 4055), *Citrobacter amalonaticus* (CCT 4059), *Pseudomonas fluorescens* (CCT 4056), *Klebsiella ozaenae* (CCT 4061) *Azotobacter vinelandii* (IZ 48) and *Serratia rubidaea* (CCT 5742), the last one was isolated from a coconut fruit, and stereoselectively reduced the 4-alkyl and 2-alkylcyclohexanones into 4-alkyl and 2-alkyl cyclohexanols in ca. 100% conversion.

Herewith, we report the diastereo- and enantioseletive bioreductions of 4-*tert*-butylcyclohexanone (**1**), 4-methylcyclohexanone (**2**) and 2-methylcyclo-

* Corresponding author. Fax: +55-19-788-3023.
E-mail address: roseli–l@iqm.unicamp.br (R.M. De Conti).

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hexanone (**3**) (Scheme 1), by the Brazilian strain of *S. rubidaea* (CCT 5742).

2. Results and discussion

The biotransformations were conducted with resting cells mass/substrate 30/1 phosphate buffer solution (PBS) pH 7 at 30°C and 110 rpm for 48 h. **1** was quantitatively reduced by the *S. rubidaea* CCT 5742 to the less thermodynamically stable isomeric alcohol, cis-4-*tert*-butylcyclohexanol **1a**, with 96% diastereoisomeric excess. **2** and **3** were also totally reduced to the corresponding alcohols with high diastereo- and enantioselectivities (Scheme 1).

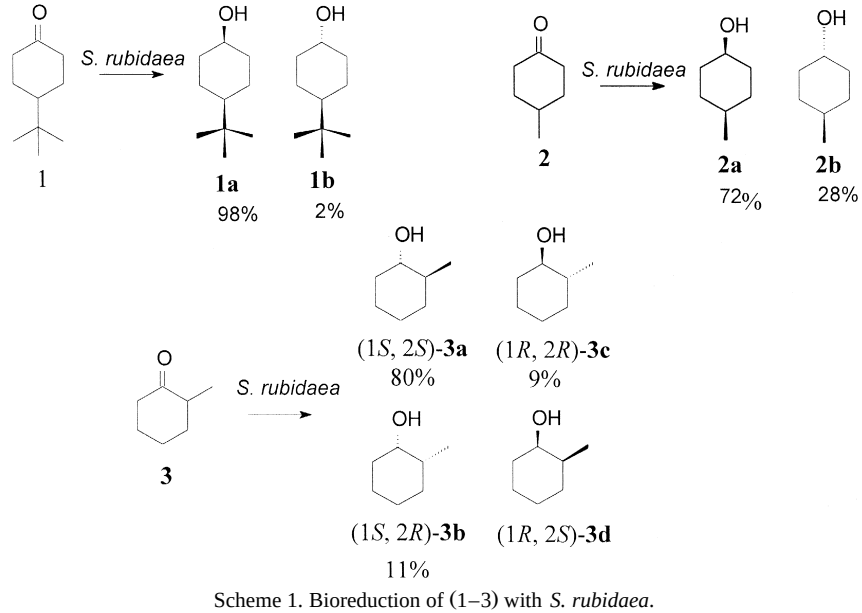
The conversion of the ketones into its corresponding alcohols was monitored by GC/MS equipped with a DB-1 fused silica capillary column. The *cis* alcohols were distinguished from their *trans* isomers by the multiplicity of their (CHOH) signals in the ^{1}H NMR (300 MHz) spectra (e.g. δ 4.0, quintet, $J = 2.7$ Hz for **1a**; δ 3.3, triplet of triplets, $J = 4.4$ and 8.9 Hz, for trans-4-*tert*-butylcyclohexanol **1b**), taking into consideration the predominant conformer of each diastereoisomer. Our results are in good agreement with previously reported data [3,8]. The de and ee of the bioreduction products were determined by the chiral (GC/FID) equipped with a Chrompack capillary column, by comparing it with the racemic *cis* and *trans* mixture obtained from the chemical reduction of ketones 1–3. The results are summarised in Table 1.

As shown in Table 1, the biological reduction of **1** and **2** with *S. rubidaea* afforded the thermodynamically less stable *cis*-isomers **1a** and cis-4-methylcyclohexanol **2a** with 96% and 44% de, respectively.

Farooq and Hanson [8] reported the biotransformation of **1** using the fungus *Cephaloporium aphidicola* that produced a mixture of 1:1 **1a**/**1b** in low yields after 10 days of incubation.

The biological enantioselective reduction of **2** with *Glomerella cingulata* produced **2a** in 100% conversion after 8 days of incubation [10]. The Brazilian strain *S. rubidaea* yielded similar results, however, in a shorter period of time (48 h).

The reduction of **3** by *S. rubidae* produced *trans*-2-methylcyclohexanols **3a** and **3c** in de of 78%. The ee of **3a** and **3c** was determined as 80% and that of the *cis*-alcohols **3b** and **3d** as 98%. The absolute configuration of the compounds was determined as (1*S*, 2*S*)-**3a**, (1S, 2*R*)-**3b**, (1*R*, 2*R*)-**3c** and (1*R*, 2*S*)-**3d** by specific optical rotation comparison of the



Scheme 1. Bioreduction of (1–3) with *S. rubidaea*.

Table 1
Diastereo- and enantiomeric excesses of the products obtained from the bioreduction of 1–3 by *S. rubidaea*

Substrates	Products	de (%)	ee (%)
1	**1a**	96 (*cis*)	–
			–
2	**2a**	44 (*cis*)	–
			–
3	**3a** + **3c**	78 (*trans*)	80
	3b + **3d**		98

pure diastereomers with the data reported by Miyazawa et al. [10].

A different behaviour was observed for the reduction of **3** by *C. cingulata* [10], which afforded mainly the *cis*-2-methylcyclohexanol **3b** and **3c** with an inverse relative and absolute configurations from the reductions obtained by *S. rubidaea*.

From these results, it was concluded that the Brazilian strain *S. rubidaea* efficiently reduced 1–3 with a simultaneous in situ epimerization of the position alpha to ketone **3** affording valuable chirons with almost 100% conversion.

3. Experimental

3.1. General procedure for the bioreductions

The bacteria were reactivated in nutrient broth (NB-nutrient broth-Difco) (50 ml) for 24 h at 30°C. The cells were transferred to an erlenmeyer (2 l) in NB (1 l) and the bacteria were incubated in a shaker at 110 rpm and 30°C for 17 h. After that, the cells were centrifuged for 30 min at 300 rpm and then transferred to erlenmeyers (250 ml) containing PBS pH 7 (100 ml). The substrates (50 mg) were dissolved in ethanol (1 ml) and added to the cells medium in cell/substrate ratio of 30:1. The mixture was incubated at 30°C and 110 rpm for 48 h. The reaction mixture was then extracted with ethyl acetate, the organic layer dried over magnesium sulphate and filtered through a celite column. The solvent was evaporated under reduced pressure and the residue dissolved in ethyl acetate (10 mg/ml) was analyzed by GC/MS with a fused silica capillary column (30 m, 0.25 mm, 1 μm) DB-5, $T_i = 80$°C, $T_f = 280$°C, $T_{inj} = 280$°C, $T_{detect} = 280$°C and GC/FID equipped with a fused silica capillary column CP-Chirasil-DEX CB (25 m, 0.25 mm, 0.25 μm), $T_i = 80$°C, $T_f = 180$°C, $T_{inj} = 250$°C, $T_{detect} = 280$°C.

cis-4-*tert*-butylcyclohexanol **1a** — (GC/FID) retention time = 21.60 min (98%), (GC/MS) retention time = 6.92, MS: *m/z* (%) 156(M^+, 1) 141(2) 123(16) 99(40) 81(51) 65(87) 57(100) 41(92). ^{1}H NMR ($CDCl_3$/TMS 300 MHz): δ 0.95 (s, 9H); 1.20–1.60 (m, 5H); 1.80–2.00 (m, 4H); 4.03 (q, 1H, $J = 2.7$ Hz).

trans-4-*tert*-butylcyclohexanol **1b** — (GC/FID) retention time = 21.00 min (2%), (GC/MS) retention time = 6.92, MS: *m/z* (%) 156(M^+, 1) 141(2) 123(16) 99(40) 81(51) 65(87) 57(100) 41(92). ^{1}H NMR ($CDCl_3$/TMS 300 MHz): δ 0.98 (s, 9H); 1.20–1.60 (m, 5H); 1.80–2.00 (m, 4H); 3.30 (tt, 1H, $J = 4.4$ and 8.9 Hz).

cis-4-methylcyclohexanol **2a** — (GC/FID) retention time = 12.26 min (72%), (GC/MS) retention time = 3.34, MS: *m/z* (%) 114(M^+, 2) 96(14) 81(46) 70(21) 57(100) 41 (48). ^{1}H NMR ($CDCl_3$/TMS 300 MHz): δ 0.95 (s, 3 H); 1.20–1.50 (m, 5H); 1.65–1.75 (m, 2H); 1.90–2.00 (m, 2H); 3.90 (q, 1H, $J = 3$ Hz).

trans-4-methylcyclohexanol **2b** — (GC/FID) retention time = 11.69 min (28%), (GC/MS) retention time = 3.34, MS: *m/z* (%) 114(M^+, 2) 96(14) 81(46) 70(21) 57(100) 41 (48). ^{1}H NMR ($CDCl_3$/TMS 300 MHz): δ 0.97 (s, 3H); 1.20–1.50 (m, 5H); 1.65–1.75 (m, 2H); 1.90–2.00 (m, 2H); 3.50 (tt, 1H, $J = 4.5$ and 11 Hz).

cis-2-methylcyclohexanol **3b** — (GC/FID) retention time = 11.64 min (11%), ee 98%, (GC/MS) retention time = 3.36, MS: *m/z* (%) 114(M^+, 2) 96(29) 81(41) 68(48) 57(100) 41(73). ^{1}H NMR ($CDCl_3$/TMS 300 MHz): δ 0.95 (d, 3H, $J = 5,7$ Hz); 1.20–1.45 (m, 4H); 1.50–1.70 (m, 4H); 1.73–1.80 (m, 1H); 3.80 (q, 1H, $J = 3$ Hz). $[\alpha]_D^{25} = +58.0°$ ($c = 7.5$, $CHCl_3$), Lit [10] $[\alpha]_D^{20} = +12.1$ ($c = 1.0$, $CHCl_3$).

trans-2-methylcyclohexanol (**3a** + **3c**) — (GC/FID) retention time = 10.88 min (89%), ee 80%, (GC/MS) retention time = 3.25, MS: *m/z* (%) 114(M^+, 3) 96(29) 81(48) 68(52) 57(100) 41(78). ^{1}H NMR ($CDCl_3$/TMS 300 MHz): δ 1.05 (d, 3H, $J = 4.5$ Hz); 1.20–1.45 (m, 4H); 1.50–1.70 (m, 4H); 1.73–1.80 (m, 1H); 3.10 (dt, 1H, $J = 4.3$

and 9.6 Hz). $[\alpha]_D^{25} = +10{,}0°$ ($c = 7.5$, $CHCl_3$), Lit [10] $[\alpha]_D^{20} = +2.0$ ($c = 1.0$, $CHCl_3$).

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