DOI: 10.1002/adsc.200606055

Biocatalytic Racemization of α-Hydroxy Ketones (Acyloins) at Physiological Conditions using *Lactobacillus paracasei* DSM 20207

Bettina M. Nestl,[a,b] Wolfgang Kroutil,[a] Kurt Faber[a,*]

[a] Department of Chemistry, Organic and Bioorganic Chemistry, University of Graz, Heinrichstrasse 28, 8010 Graz, Austria
Fax: (+43)-316-380-9840, phone: (+43)-316-380-5332, e-mail: Kurt.Faber@Uni-Graz.at
[b] Research Centre Applied Biocatalysis, 8010 Graz, Austria

Received: February 10, 2006; Accepted: March 17, 2006

Supporting Information for this article is available on the WWW under http://asc.wiley-vch.de/home/.

Abstract: Biocatalytic racemization of open-chain and cyclic dialkyl-, alkyl-aryl- and diaryl-substituted acyloins was accomplished using whole resting cells of *Lactobacillus paracasei* DSM 20207. The mild (physiological) reaction conditions ensured the suppression of undesired side reactions, such as elimination or condensation. This novel biocatalytic isomerization protocol represents an essential tool for the deracemization of pharmacologically important building blocks.

Keywords: acyloins; biotransformations; *Lactobacillus*; racemization

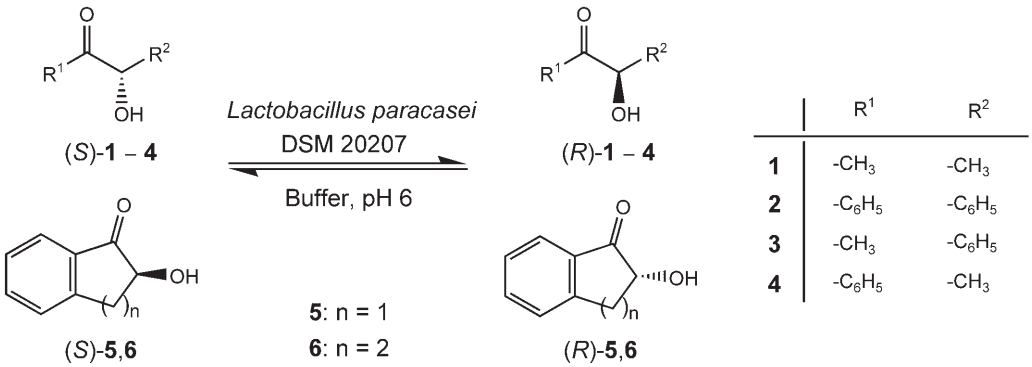
Until recently, the entropy-driven isomerization of enantiomers – racemization – was generally considered as an unwanted side reaction rather than a synthetically useful transformation. As a consequence, the controlled racemization of organic compounds has been studied rather scarcely and the number of protocols available to date using mild reaction conditions is very small.[1] The importance of clean racemization processes for organic synthesis predominantly emerged from their key role in so-called deracemization processes,[2] which allow the transformation of a racemate into a single stereoisomeric product without the occurrence of an unwanted stereoisomer in 100% theoretical yield. Since the majority of chemical racemization protocols require harsh reaction conditions, they are incompatible with an (*in-situ*) enantioselective biocatalytic transformation. In contrast, enzymes are highly compatible with each other and biocatalytic racemization thus holds great potential.[3]

We have recently shown that mandelate racemase [EC 5.1.2.2] from *Pseudomonas putida* ATCC 12633 is an excellent catalyst for the isomerization of a wide spectrum of β,γ-unsaturated α-hydroxycarboxylic acids.[4] However, saturated (aliphatic) analogues were not accepted at all. This limitation was successfully overcome by the use of whole resting cells of *Lactobacillus* spp., which allowed the clean racemization of a wide range of aliphatic and aryl-aliphatic α-hydroxycarboxylic acids.[5]

In order to broaden the portfolio of biocatalytic racemization, we envisaged to extend the substrate range towards other hydroxy-compounds – acyloins. Non-racemic α-hydroxy ketones[6] constitute popular building blocks for the synthesis *vic*-diols and amino alcohols through diastereoselective reduction/alkylation and reductive amination, respectively, with highly efficient chirality transfer.[7] Chemical racemization of acyloins usually proceeds through the corresponding enediol and thus predominantly features methods based on acid- or base-catalysis.[8] The latter are often plagued by elimination as a major side reaction due to the ease of formation of a resonance-stabilized conjugated enone. Our search for a biocatalytic racemization of acyloins was triggered by the speculative (but unproven) existence of an acetoin racemase[9] and benzoin racemase, respectively.[10]

In order to cover a reasonably broad range of substrates, enantiopure acyloins **1**–**6** bearing small and large groups at both ends were selected for our initial tests using rehydrated lyophilized (resting) cells of *Lactobacillus paracasei* DSM 20207 (Scheme 1, Table 1) in aqueous buffer at pH 6.

In order to obtain quantitative values of activities for reasons of comparison, relative rates of racemization were calculated from the slope of initial progress curves of the decline of ee versus time at a conversion of $<5\%$ (Table 1). The smallest substrate [acetoin (*R*)-**1**] was rapidly racemized and its activity was arbitrarily set as standard (100%). Isomerization of the bulky substrate



Scheme 1. Biocatalytic racemization of acyloins **1**–**6**.

Table 1. Relative racemization rates of acyloins **1**–**6**.

Substrate	R^1	R^2	Time [h]	ee [%]	Time [h]	ee [%]	Relative Rate [%][a]
(*R*)-**1**	$-CH_3$	$-CH_3$	6	19	48	6	100
(*R*)-**2**	$-C_6H_5$	$-C_6H_5$	6	72	72	30	56
(*S*)-**2**	$-C_6H_5$	$-C_6H_5$	6	35	72	4	83
(*R*)-**3**	$-CH_3$	$-C_6H_5$	6	58	72	10	67
(*R*)-**4**	$-C_6H_5$	$-CH_3$	6	55	72	11	65
(*R*)-**5**	n = 1		6	68	72	20	58
(*R*)-**6**	n = 2		6	45	72	6	77

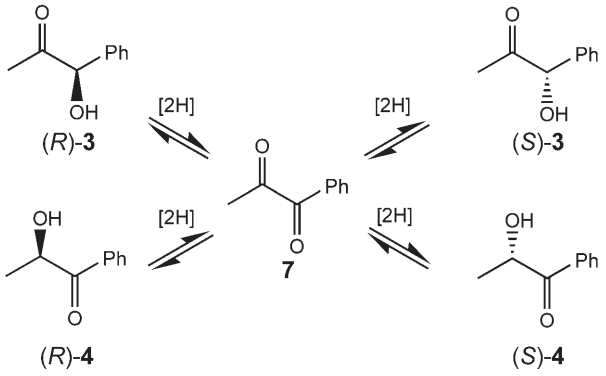
[a] Relative rates were calculated from initial progress curves at a conversion of $<5\%$, the racemization rate of substrate (*R*)-**1** was arbitrarily set as standard (100%).

benzoin (**2**) proceeded surprisingly well. Detailed analysis using both stereoisomers revealed that the (*S*)-enantiomer (83%) was racemized more rapidly than the (*R*)-counterpart (56%). Such "non-symmetrical" kinetics are not uncommon in enzyme-catalyzed racemization and are caused by differences in k_{cat} and K_M values of enantiomers.[5a,11] It should be stressed that racemization of both of the latter substrates proved to be essentially "clean" and no trace of side-products likely to arise from elimination, oxidation, condensation or rearrangement could be found within the detection limits.

Although the racemization of the aryl-alkyl derivatives (*R*)-**3** ("phenyl-acetylcarbinol", PAC) and (*R*)-**4** proceeded sufficiently rapidly (*ca.* 66% relative to acetoin), traces of side products arising from redox reactions were detected: In both cases, dione **7** was formed in *ca.* 2–3% and the respective "crossover-compounds," i.e., (*S*)-**4** in the case of substrate (*R*)-**3** and *vice versa*, were detected in low amounts (2–3%). The formation of these side-products strongly suggests that the racemization predominantly takes place through an equilibrium-controlled biocatalytic reduction-oxidation sequence[12,13] rather than through involvement of a single "racemase" enzyme (Scheme 2).[9,10]

L-PAC (*R*)-**3** and derivatives thereof are key intermediates for the industrial manufacture of (−)-(pseudo)ephedrine, anti-depressants and smoking cessation agents *via* a reductive amination.[7,14]

The clean racemization of cyclic acyloins is of special interest, since these compounds are rather unstable and tend towards elimination forming highly stabilized conjugated enones.[15] To our delight, both cyclic acyloins (*R*)-**5** and (*R*)-**6** were quickly racemized (relative rates 58 and 77%, respectively) in a clean fashion: neither the corresponding diols, diones nor (most importantly) enones could be detected. 2-Hydroxy-1-indanone (*R*)-**5** is a key intermediate for the synthesis of several HIV protease inhibitors, such as Indinavir and derivatives thereof,[16] used in the therapy of AIDS.[17] The existence of a racemase (within whole cells of the



Scheme 2. Biocatalytic isomerization of (*R*)-**3** and (*R*)-**4** *via* reduction-oxidation through dione **7**.

yeast *Trichosporon cuntaneum* MY 1506) acting on substrate **5** was initially postulated, but later disproven.[18]

In summary, biocatalytic racemization of a range of structurally diverse linear and cyclic acyloins was accomplished for the first time using whole (resting) cells of *Lactobacillus paracasei* DSM 20207. Due to the mild (physiological) reaction conditions, isomerizations proved to be "clean" and side products were detected only in trace amounts, if any. The fact that only marginal racemization took place using heat-deactivated cells proved the enzymatic character of this isomerization.

The enzyme(s) involved in this biotransformation and their substrate tolerance are currently being investigated in detail. These results will allow us to extend our recently developed one-pot two-enzyme deracemization strategy[19] towards acyloins.

Experimental Section

General Procedure for the Biocatalytic Racemization of Acyloins

Lyophilized cells (50 mg) were rehydrated in BIS-TRIS buffer (0.5 mL, 50 mM, 10^{-2} M $MgCl_2$, pH 6) for 1 h at 42 °C with shaking at 150 rpm. Substrate **1**–**6** (5 mg) was added followed by shaking of the reaction mixture at 150 rpm and 42 °C. After a given time, the cells were removed by centrifugation. The supernatant was extracted with ethyl acetate and the organic phase was dried over sodium sulfate. The determination of conversion and the enantiomeric excess was carried out by HPLC on a chiral stationary phase (see Supporting Information, Table S1). For HPLC analysis, the organic phase was evaporated under reduced pressure and the residue was dissolved in HPLC eluent.

Supporting Information

For the synthesis of substrates, determination of absolute configuration by optical rotation, preparation of biocatalyst, and analytical procedures see the supporting information.

Acknowledgements

This study was performed within the Research Centre Applied Biocatalysis in cooperation with BASF AG (Ludwigshafen) and financial support by the FFG, the City of Graz and the Province of Styria is gratefully acknowledged. B. Hauer and R. Stürmer (BASF AG) are cordially thanked for their valuable contributions.

References and Notes

[1] E. J. Ebbers, G. J. A. Ariaans, J. P. M. Houbiers, A. Bruggink, B. Zwanenburg, *Tetrahedron* **1997**, *53*, 9417–9476.

[2] K. Faber, *Chem. Eur. J.* **2001**, *7*, 5004–5010; O. Pamies, J.-E. Baeckvall, *Chem. Rev.* **2003**, *103*, 3247–3261.

[3] B. Larissegger-Schnell, K. Faber, W. Kroutil, *Adv. Synth. Catal*. **2003**, *345*, 653–666; M. E. Tanner, *Acc. Chem. Res.* **2002**, *35*, 237–246.

[4] U. Felfer, M. Goriup, M. F. Koegl, U. Wagner, B. Larissegger-Schnell, K. Faber, W. Kroutil, *Adv. Synth. Catal.* **2005**, *347*, 951–961.

[5] a) S. M. Glueck, B. Larissegger-Schnell, K. Csar, W. Kroutil, K. Faber, *Chem. Commun*. **2005**, 1904–1905; b) S. M. Glueck, M. Pirker, B. M. Nestl, B. T. Ueberbacher, B. Larissegger-Schnell, K. Csar, B. Hauer, R. Stuermer, W. Kroutil, K. Faber, *J. Org. Chem*. **2005**, *70*, 4028–4032.

[6] M. Müller, *Angew. Chem. Int. Ed.* **2005**, *44*, 362–365; A. S. Demir, O. Sesenoglu, E. Eren, B. Hosrik, M. Pohl, E. Janzen, D. Kolter, R. Feldmann, P. Dünkelmann, M. Müller, *Adv. Synth. Catal.* **2002**, *344*, 96–103; M. Pohl, B. Lingen, M. Müller, *Chem. Eur. J.* **2002**, *8*, 5288–5295.

[7] A. S. Demir, H. Hamamci, O. Sesenoglu, F. Aydogan, D. Capanoglu, R. Neslihanoglu, *Tetrahedron: Asymmetry*, **2001**, *12*, 1953–1956; A. J. Smallridge, M. A. Trewhalla, A. Maurice, A. K. Wilkinson, *WO Patent* 2003018531A1 20030306, *Chem. Abstr.* **2003**, *138*, 205242.

[8] a) T. Taniguchi, K. Ogasawara, *Chem. Commun.* **1997**, 1399–1400; b) S. Easwar, S. B. Desai, N. P. Argade, K. N. Ganesh, *Tetrahedron: Asymmetry*, **2002**, *13*, 1367–1371; c) P. Ödman, L. A. Wessjohann, U. T. Bornscheuer, *J. Org. Chem.* **2005**, *70*, 9551–9555; d) A. S. Demir, H. Hamamci, P. Ayhan, A. N. Duygu, A. C. Igdir, D. Capanoglu, *Tetrahedron: Asymmetry* **2004**, *15*, 2579–2582.

[9] M. Voloch, M. R. Ladisch, V. W. Rodwell, G. T. Tsao, *Biotechnol. Bioeng.* **1983**, *25*, 173–183.

[10] G. J. A. Conceicao, P. J. S. Moran, J. A. R. Rodrigues, *Tetrahedron: Asymmetry* **2003**, *14*, 2327–2330.

[11] M. A. Spies, J. J. Woodward, M. R. Watnik, M. D. Toney, *J. Am. Chem. Soc.* 2004, **126**, 7464–7475; K. Shibata, K. Shirasuna, K. Motegi, Y. Kera, H. Abe, R. Yamada, *Comp. Biochem. Physiol. B*, 2000, **126**, 599–608.

[12] W. Hummel, M.-R. Kula, *Eur. J. Biochem.* **1989**, *184*, 1–13; for a detailed mechanistic investigation on the biocatalytic racemization of α-hydroxy carboxylic acids, see: B. M. Nestl, S. M. Glueck, B. Hauer, R. Stuermer, W. Kroutil, K. Faber, manuscript in preparation.

[13] For a mechanistically related interconversion of benzoin enantiomers by microbial stereo-inversion mediated by whole fungal cells see ref.[8d]

[14] B. Rosche, M. Breuer, B. Hauer, P. L. Rogers, *Biotechnol. Bioeng.* **2004**, *86*, 788–794; S. Engel, M. Vyazmensky, S. Geresh, Z. Barak, D. M. Chipman, *Biotechnol. Bioeng.* **2003**, *83*, 833–640.

[15] Elimination is extremely facile and was even promoted by exposure to neutral silica gel during column chromatography.

[16] Y. Cheng, F. Zhang, T. A. Rano, Z. Lu, W. A. Scheleif, L. Gabryelski, D. B. Olsen, M. Stahlhunt, C. A. Rutkowski, J. H. Lin, L. Jin, E. A. Emini, K. T. Chapman, J. R. Tata, *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2419–2422.

[17] I. Lunardi, G. J. A. Conceicao, P. J. S. Moran, J. A. R. Rodrigues, *Tetrahedron: Asymmetry* **2005**, *16*, 2515–2519.
[18] G. J. A. Conceicao, P. J. S. Moran, J. A. R. Rodrigues, *Chem. Listy* **2003**, *97*, 473; T. Cazetta, G. J. A. Conceicao, I. Lunardi, P. J. S. Moran, J. A. R. Rodrigues, poster # PS-003 at *BMOS-11*, Canela, Brazil, August 29–September 02, 2005; (http://coralx.ufsm.br/bmos/poster session.php).
[19] B. Larissegger-Schnell, W. Kroutil, K. Faber, *Synlett*. **2005**, 1936–1938; B. Larissegger-Schnell, S. M. Glueck, W. Kroutil, K. Faber, *Tetrahedron* **2006**, *62*, 2912–2916.

