# A "Designer Yeast" That Catalyzes the Kinetic Resolutions of 2-Alkyl-Substituted Cyclohexanones by Enantioselective Baeyer−Villiger Oxidations

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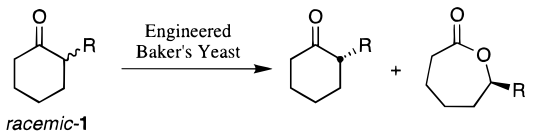
Optically active lactones are valuable intermediates in asymmetric synthesis. In particular, 6-substituted $\epsilon$-caprolactones are especially useful, and several methods for producing these compounds have been explored. These include the chromatographic separation of diastereomeric precursors,[1] Baeyer−Villiger oxidations of optically-enriched 2-substituted cyclohexanones,[2,3] the use of esterases to enantioselectively hydrolyze the racemic lactones,[4−6] and the development of metal complexes that perform enantioselective Baeyer−Villiger oxidations on the corresponding substituted cyclohexanones.[7,8] Unfortunately, these methods are either experimentally cumbersome or afford lactones with only modest enantioselectivities.

Since racemic 2-alkyl-substituted cyclohexanones are readily available, a Baeyer−Villiger oxidation that effected a kinetic resolution of these ketones would be an attractive strategy (Scheme 1). Enzymes that perform enantioselective Baeyer−Villiger oxidations with broad substrate specificities have been isolated from several microbial species including *Cylindrocarpon destructans*,[9] *Pseudomonas putida*,[10−12] and *Acinetobacter*.[13] Here, we describe an experimentally simple method for producing 6-substituted $\epsilon$-caprolactones in high optical purities using whole cells of "oxidizing yeast"—a strain of bakers' yeast that has been engineered to express *Acinetobacter* sp. NCIB 9871 cyclohexanone monooxygenase.[14]

**Scheme 1**




*racemic*-**1**

**a** R = Me
**b** R = Et
**c** R = *n*-Pr
**d** R = *i*-Pr
**e** R = *n*-Bu
**f** R = allyl

Our expectation that the *Acinetobacter* monooxygenase would provide a kinetic resolution of 2-alkyl-substituted cyclohexanones was based on the earlier work of Furstoss[15,16] and Schwab, who demonstrated that 2-methylcyclohexanone was oxidized by the enzyme to the corresponding lactone with modest enantioselectivity.[17] Interestingly, despite the large number of ketones that have been tested as substrates for this enzyme, simple 2-substituted cyclohexanones have not been systematically investigated, and only a single example (2-methylcyclohexanone) has been reported.[17] Reasoning that larger substituents at the 2-position might lead to greater enantioselectivities, we tested a number of racemic cyclohexanones as substrates for this enzyme, and our results are summarized in Table 1.[18−20] All of the reactions were performed by the engineered bakers' yeast using the previously-described procedure.[14,21] Because several of these substrates were insoluble in the aqueous growth medium, stoichiometric amounts of $\beta$-cyclodextrin were included in the reaction mixture.[22] Chiral-phase GC provided the enantiomeric compositions of both the ketone and lactone during the course of the reaction. As noted previously,[14] ketone reduction was only a minor side reaction. In addition, the olefin functionality was inert in the yeast-mediated oxidation of **1f**. For preparative purposes, the reactions were terminated shortly after

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(1) Pirkle, W. H.; Adams, P. E. *J. Org. Chem.* **1979**, 44, 2169−2175.
(2) Matsumoto, K.; Tsutsumi, S.; Ihori, T.; Ohta, H. *J. Am. Chem. Soc.* **1990**, 112, 9614−9619.
(3) Katoh, O.; Sugai, T.; Ohta, H. *Tetrahedron: Asymmetry* **1994**, 5, 1935−1944.
(4) Blanco, L.; Guibé-Jampel, E.; Rousseau, G. *Tetrahedron Lett.* **1988**, 1915−1918.
(5) Fouque, E.; Rousseau, G. *Synthesis* **1989**, 661−666.
(6) Fellous, R.; Lizzani-Cuvelier, L.; Loiseau, M. A.; Sassy, E. *Tetrahedron: Asymmetry* **1994**, 5, 343−346.
(7) Bolm, C.; Schlingloff, G.; Weickhardt, K. *Angew. Chem., Int. Ed. Engl.* **1994**, 33, 1848−1849.
(8) Gusso, A.; Baccin, C.; Pinna, F.; Strukul, G. *Organometallics* **1994**, 13, 3442−3451.
(9) Königsberger, K.; Braunegg, G.; Faber, K.; Griengl, H. *Biotechnol. Lett.* **1990**, 12, 509−514.
(10) Gagnon, R.; Grogan, G.; Levitt, M. S.; Roberts, S. M.; Wan, P. W. H.; Willetts, A. J. *J. Chem. Soc., Perkin Trans. 1* **1994**, 2537−2543.
(11) Grogan, G.; Roberts, S.; Wan, P.; Willetts, A. *Biotech. Lett.* **1993**, 15, 913−918.
(12) Adger, B.; Bes, M. T.; Grogan, G.; McCague, R.; Pedragosa-Moreau, S.; Roberts, S. M.; Villa, R.; Wan, P. W. H.; Willetts, A. J. *J. Chem. Soc., Chem. Commun.* **1995**, 1563−1564.
(13) Donoghue, N. A.; Norris, D. B.; Trudgill, P. W. *Eur. J. Biochem.* **1976**, 63, 175−192.
(14) Stewart, J. D.; Reed, K. W.; Kayser, M. M. *J. Chem. Soc., Perkin Trans. 1* **1996**, 755−758.
(15) Alphand, V.; Archelas, A.; Furstoss, R. *Biocatalysis* **1990**, 3, 73.
(16) Alphand, V.; Archelas, A.; Furstoss, R. *J. Org. Chem.* **1990**, 55, 347−350. For a more exhaustive compilation of references describing the application of cyclohexanone monooxygenase to organic synthesis, see ref 14 and references therein.
(17) Schwab, J. M.; Li, W.-b.; Thomas, L. P. *J. Am. Chem. Soc.* **1983**, 105, 4800−4808.
(18) Ketones that were not commercially available were prepared by alkylation of the morpholine enamine of cyclohexanone: Stork, G.; Brizzolara, A.; Landesman, H.; Szmuszkovicz, J.; Terrell *J. Am. Chem. Soc.* **1963**, *85*, 207−221. While many ketones could be successfully oxidized by our "designer yeast", this reagent was unable to oxidize either 2-benzyl- or 2-isobutylcyclohexanone since these hydrophobic compounds prevented growth of the cells, even in the presence of cyclodextrins.
(19) Saigo, K.; Kasahara, A.; Ogawa, S.; Nohira, H. *Tetrahedron Lett.* **1983**, 511−512.
(20) Kim, H.-d.; Kawasaki, H.; Nakajima, M.; Koga, K. *Tetrahedron Lett.* **1989**, 6537−6540.
(21) Typical experimental procedure: A 0.20 g portion of frozen, washed 15C(pKR001) cells was added to 100 mL of YEP-galactose (1% Bacto-yeast extract, 2% Bacto-peptone, 2% galactose) along with 0.10 g (0.78 mmol) of 2-allylcyclohexanone and 0.70 g of $\beta$-cyclodextrin. The culture was shaken at 200 rpm at 30 °C and sampled periodically for GC analysis. After half of the substrate had been consumed (approximately 20 h after the start of fermentation), the cells were removed by centrifuging at 4000*g* for 10 min at 4 °C. The supernatant was extracted with $CH_2Cl_2$ (4 × 50 mL), and then the combined organic extracts were dried ($MgSO_4$), filtered, and evaporated. The residue was chromatographed over silica gel using 1:1 ether:hexanes as the eluant to afford 29 mg of the (*S*)-ketone (58% yield) and 44 mg of the (*R*)-lactone (78% yield).
(22) (a) Bar, R. *Trends Biotechnol.* **1989**, 7, 2−4. (b) In general, 1 equiv of $\beta$-cyclodextrin was sufficient to solubilize the ketones in the aqueous growth medium; however, the biotransformation of 2-isopropylcyclohexanone required 2.0 equiv of hydroxypropyl-$\beta$-cyclodextrin to afford a homogeneous mixture.

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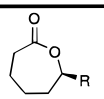
**Table 1. Kinetic Resolutions of Racemic 2-Alkylcyclohexanones by Whole Cells of Bakers' Yeast That Express Cyclohexanone Monooxygenase**

|  |  | | | |  | | | | *E* | Ref. |
|---|---|---|---|---|---|---|---|---|---|---|
| | Yield[a] | | %ee[b] | $[\alpha]_D$[c] | Yield | | %ee | $[\alpha]_D$ | | |
| **a**, R = Me | --- | (*R*) | --- | --- | 50% | (*S*) | 49 | -6.5° *c* 1.8 | 10[d] | 5 |
| **b**, R = Et | 69% | (*R*) | ≥ 98 | -24° *c* 3.0 | 79% | (*S*) | 95 | -37° *c* 1.8 | ≥ 200 | 19 |
| **c**, R = *n*-Pr | 66% | (*R*) | 92 | -15° *c* 1.6 | 54% | (*S*) | 97 | -32° *c* 1.4 | ≥ 200 | 4 |
| **d**, R = *i*-Pr | 46% | (*S*) | 96 | -49.2° *c* 2.03 | 41% | (*R*) | ≥ 98 | -15.0° *c* 2.02 | ≥ 200 | 20 |
| **e**, R = *n*-Bu | 64% | (*R*) | 98 | -29.4° *c* 1.17 | 59% | (*S*) | ≥ 98 | -18.6° *c* 1.11 | ≥ 200 | 6 |
| **f**, R = allyl | 58% | (*S*) | ≥ 98 | -10° *c* 1.6 | 59% | (*R*) | ≥ 98 | -22° *c* 2.2 | ≥ 200 | 19 |

[a] Yields refer to chromatographically purified samples.
[b] Values of enantiomeric excess were determined by chiral-phase GC analysis on a Chirasil-Dex CB column.
[c] Optical rotations were measured in $CHCl_3$ solutions at ambient temperature.
[d] The preference for the (*S*)-ketone isomer was confirmed by the sign of the specific rotation obtained from the lactone product isolated from a reaction that was allowed to proceed to 60% completion.

50% conversion and the lactone and the remaining ketone were isolated by silica gel chromatography. In all cases, the "normal" Baeyer−Villiger regioisomer was obtained and the (*S*)-ketone was the more reactive enantiomer.[23] The absolute configurations of the products were determined by comparing the optical rotations of the ketones or the corresponding lactones to literature values.[4−6,19,20]

In general, the optical purities of both the lactones and the remaining ketones were extremely high, making this method attractive for preparative purposes. The enantioselectivity can be quantitatively described by *E*, the ratio of the second-order rate constants for the conversion of the substrate enantiomers to the two-product enantiomers, a ratio that is independent of enzyme concentration or mass transport phenomena (Figure 1).[24] For a kinetic resolution to be preparatively useful, *E* should be >30. In the case of cyclohexanone monooxygenase, all but one of the *E* values were ≥200, which represents essentially complete enantioselectivity. For this reason, it was possible to isolate both the remaining ketone and the lactone in very high optical purities from a single reaction. The sole exception was the *E* value of 10 for 2-methylcyclohexanone. Presumably, the steric size of the substituent is insufficient to completely enforce the stereochemical imperative in this case. Because the nonenzymatic Baeyer−Villiger oxidation proceeds with retention of stereochemistry, the optically pure ketones obtained from the biotransformations can be converted to the corresponding lactones by peracid oxidation. Thus, both lactone enantiomers can be produced from the racemic ketone. For example, racemic **1b** was converted by our "designer yeast" into (*R*)-**1b** (94% ee, 69% yield) and (*S*)-**2b** (98% ee, 79% yield). The recovered ketone was then oxidized by *m*-CPBA to afford (*R*)-**2b** in 55% overall yield and 94% ee.

This work provides a useful example of the very high enantioselectivities that can be achieved by applying enzyme catalysis to organic synthesis. Furthermore, by expressing the enzyme in bakers' yeast, we have created a simple *reagent* that can be used by chemists with no training in biochemistry or microbiology. This approach eliminates the need to isolate the enzyme and sidesteps problems associated with overmetabolism of the lactone products. Yeast-mediated reactions are environmentally compatible and do not product toxic byproducts, making them ideal for large-scale applications.

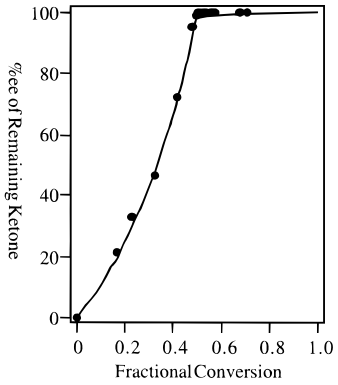



**Figure 1.** Yeast-mediated Baeyer−Villiger oxidation of **1b**. The reaction mixture contained 10 mM ketone and 0.20 g of washed yeast cells in 100 mL of growth medium. The mixture was shaken at 200 rpm (30 °C) and sampled periodically. Chiral-phase GC analysis was used to determine the enantiomeric excess values for the remaining ketone and the lactone product. To determine the value of *E* , the enantiomeric excess of the remaining starting ketone as a function of fractional conversion was fit to an equation derived from the model of Sih with *E* as the only adjustable parameter. The calculated line (*E* = 200) is superimposed on the data to show the quality of the fit.

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**Supporting Information Available:** Procedures for preparing the yeast cells and chiral-phase GC analyses of the reaction products (13 pages).

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(23) Note that the (*R*/*S*) designation is reversed for **1d**, **2d**, **1f**, and **2f** as a result of a change in priority numbers for the substituents.
(24) Chen, C.-S.; Fujimoto, Y.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1982**, 104, 7294−7299.