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Highly efficient extractive biocatalysis in the asymmetric reduction of an acyclic enone by the yeast *Pichia stipitis*

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Abstract—A concise highly chemo- and enantioselective preparation of (*S*)-2-ethyl-1-phenylprop-2-en-1-ol **6** (65% yield, >99% e.e.) via extractive biocatalysis by the yeast *Pichia stipitis* CCT 2617-mediated reduction of 2-ethyl-1-phenylprop-2-en-1-one **5** adsorbed on Amberlite™ XAD-7 is reported. © 2003 Published by Elsevier Science Ltd.

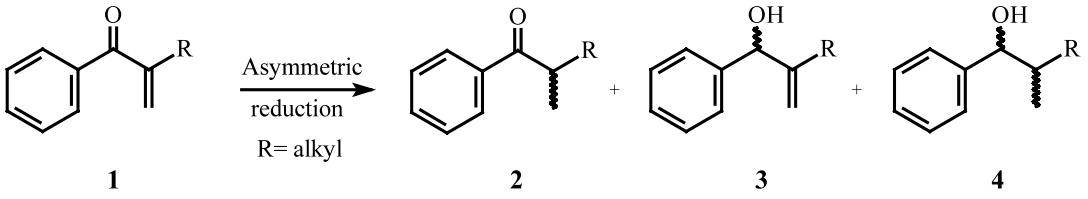
Enantiomerically pure allylic alcohols are valuable synthetic intermediates and many useful stereoselective transformations can be performed by taking advantage of the 1,2- or 1,3-allylic strain in such systems.[1] Most syntheses of chiral allylic alcohols rely on the resolution of racemic alcohols by Sharpless' asymmetric epoxidation[2] or enzyme-mediated processes.[3] As such, known examples of efficient chemo- and enantioselective reductions of α-methyleneketones **1** (Scheme 1) are scarce.[4]

Currently, much emphasis has been given to obtain enantiomerically pure compounds in good yields under environmentally friendly conditions.[5] As a result, biocatalysis with either enzymes (crude or isolated) or whole cells has blossomed over the past few years as a viable alternative to costly or difficult chemistry in the manufacture of chiral compounds.[6] Biocatalysts display wide substrate acceptance and can catalyse chemo-, regio- and stereoselective transformations of organic compounds under very mild and environmentally safe reaction conditions.[7]

In a previous work, we reported our attempts to set up stereoselective reductions of a large number of acyclic α-methyleneketones using Baker's yeast as biocatalyst.[4a] It was found that substrates, such as **1** (Scheme 1) could be unselectively reduced at the C–C double bond to afford ketone **2**, but the C=O bond was not reduced at all. We felt that a screening of microorganisms coupled to the modification of the reaction conditions would afford alternatives to circumvent the drawbacks faced with Baker's yeast-mediated reduction of acyclic enones.

Of the non-conventional yeasts tested,[8,9] the non-fermenting yeast *Pichia stipitis* CCT 2617 was able to reduce (partially) the enone **5** (Scheme 2), to afford a mixture of products. However, further assays (at analytical scale) pointed out that this enone was a very toxic xenobiotic to the yeast at concentrations of >80 mg L^{-1}, thus limiting the degree of conversion of the substrate and the yield of the reaction.

Among the alternatives currently available to circumvent such limitations, we chose the strategy of



Scheme 1.

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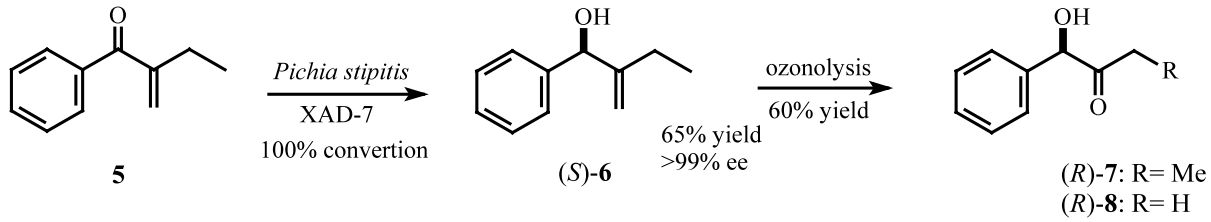

Scheme 2.

adsorbing the substrate onto a hydrophobic polymeric resin (mainly Amberlite™ XAD-type resins) as a measure to reduce and control the concentration of both substrate and product in the aqueous phase.[10]

Enone **5** was prepared as previously reported, via Mannich reaction.[8] Racemic allylic alcohol **6** for GC/MS analysis was accessed by chemoselective reduction of **5** via Luche's method.[11] Enone **1**/XAD-7 mix[12] was added to a slurry of *P. stipitis* CCT 2617[13] and the reaction was performed for 3 days.[14] This moderately nonpolar resin was chosen since it has the desirable property of roughly equal affinity for both product and substrate, driving the reaction to completion while at the same time extracting the product away from the biocatalyst effectively.

At the end of the reaction (complete consumption of **5**), the resin was filtered off and the beads were extracted with ethyl acetate. Compound **6** was obtained as the sole product with an improved isolated yield of 65% besides excellent stereocontrol (>99% e.e., Scheme 2).[15] Ozonolysis of **6** afforded acyloin **7** (60% yield).[16] The CD spectrum of **7** was closely related to that of L-phenylacetyl carbynol (L-PAC, **8**) with known *R* configuration.[17] The absolute configuration of **7** was assigned as *R*, and therefore alcohol **6** must bear *S* configuration.

This hydrophobic polymer method is based on the fact that an adsorbed hydrophobic organic compound is partitioned into the solid organic phase and the aqueous phase, the organic compound being largely in the solid phase. Thus, the polymer acts as a reservoir for the substrate, supplying by an equilibrium process the mass of substrate consumed by the biocatalyst in the aqueous phase. In turn, the product released by the biocatalyst in the aqueous phase is continuously extracted in situ by the same polymer preventing its accumulation in the aqueous phase and cell surfaces (which could cause cell death or enzyme inhibition).

In brief, the use of Amberlite™ XAD-7 proved to be an efficient strategy to circunvent the inhibitory effect of the substrate over the biocatalyst. The low concentration of the substrate in the aqueous phase drove the reaction to completion and a single product was delivered with high chemo- and enantioselectivity.

Acknowledgements

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12. Commercially available resin XAD-7 was successively washed with distilled water and acetone and then dried under reduced pressure. The substrate (200 mg) was dilluted in ethyl acetate (10 mL) and added to the treated resin (3 g). The mixture was shaken and the solvent was evaporated to dryness at reduced pressure. The solid so obtained was poured directly into the reaction vessel.
13. *Pichia stipitis* CCT 2617 stored at 'André Tosello' Research Foundation was collected in the brazilian rain forest and was cultivated in YM (yeast–malt extract) nutrient broth (1 L) for 2 days incubation at 30°C on an orbital shaker (150 rpm) before use.
14. **Typical procedure**: To a slurry of growing *Pichia stipitis* (300 mL), **5** (200 mg) adsorbed onto XAD-7 (3 g) was added. The resulting suspension was stirred in an orbital shaker (150 rpm) at 30°C until full conversion of **5** (3 days). The resin was filtered off and the beads were extracted with ethyl acetate to afford (*S*)-**6** in 65% yield after purification.
15. **Data for (*S*)-6**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.99 (t, 3H, $J=7.3$ Hz), 1.81–2.01 (m, 2H), 2.08 (s, 1H), 4.97 (s, 1H), 5.14 (s, 1H), 5.25 (m, 1H), 7.20–7.40 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.1, 24.5, 77.4, 108.6, 126.5, 127.5, 128.2, 142.1, 152.4; IR (film) 3396, 3031, 2966, 2938, 2882, 1648, 1493, 1453, 1022, 903, 844, 763, 700 cm^{-1}; $[\alpha]_D^{20}=-40$ (*c* 2, $CHCl_3$); MS *m*/*z* 162 ($M^{+\bullet}$, 14%), 147 (4%), 133 (71%), 129 (24%), 115 (21%), 107 (42%), 105 (50%), 91 (21%), 79 (100%), 77 (92%), 55 (93%), 51 (63%), 41 (29%); HRMS *m*/*z* found: 162.1043. Calcd for $C_{11}H_{14}O$: 162.1045.
16. **Data for (*R*)-7**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.00 (t, 3H, $J=7.3$ Hz), 1.26 (s, 1H), 2.36 (m, 2H), 5.10 (s, 1H), 7.25–7.40 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 7.6, 31.1, 79.4, 127.3, 128.6, 128.9, 138.2, 210.0; IR (film) 3460, 3088, 3064, 3033, 2980, 2940, 2880, 1715, 1598, 1494, 14521269, 1093, 1024, 756, 701 cm^{-1}; $[\alpha]_D^{20}=-325$ (*c* 2, $CHCl_3$); MS *m*/*z* 164 ($M^{+\bullet}$, 1%), 107 (100%), 105 (13%), 91 (92%), 79 (82%), 77 (56%), 65 (1%). HRMS *m*/*z* found: 164.0833. Calcd for $C_{10}H_{12}O_2$: 164.0837.
17. **L-PAC (*R*)-8**: (>95% e.e.) was prepared in our laboratory by baker's yeast mediated reduction of the corresponding α-diketone.