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Effect of different strains of yeast on stereocontrolled reduction of 5-acetylisoxazolines☆

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Abstract

The stereocontrolled reduction of 3-aryl-5-acetylisoxazolines (**1**) to the corresponding alcohols (**2** and **3**) in the presence of four different yeast strains, recognized as Baker's yeast (commercial), *Candida krusei* (ATCC 14243), *Pichia farinosa* (NRRL Y110) and *Sacchromyces* sp. (soil isolate) have been attempted. The *C. krusei* was found to be diastereoselective for the (*R*)-**1** while the *Sacchromyces* sp. led to complete reduction to yield the *RS*- and *SS*-alcohol in 1:1 ratio at 10 g/L scale.

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1. Introduction

The synthesis of optically pure 2-isoxazolines has been the focus of research for various workers since these heterocycles serve as useful intermediate for the synthesis of different classes of functionalized acyclic compounds and natural products [2–11]. Both enzymatic and chemical methods have been reported to obtain such isoxazoline derivatives. The chemical methods in general utilize the chiral alkenes during the intermolecular or intramolecular 1,3-dipolar cycloaddition of nitrile oxide [3,8,9,12–15] while the enzymatic method [16,17] involves the stereocontrolled reduction of isoxazolines derivatives in the presence of Baker's yeast. In our search on spermicidal agents [18], it was desired to obtain these 2-isoxazolines in enantiomerically pure form so as to carry out further transformations to obtain the chiral analogs. It has been reported earlier [17] that the stereocontrolled reduction of 5-acetylisoxazolines with baker's yeast in 2-propanol/water mixture leads to alcohol (*S*,*S*)-**2** and ketone (*R*)-**1** in 98% *ee* with no traces of anti isomer (*R*,*S*)-**3** [16]. Unfortunately in our hands, a similar reaction condition led to the recovery of all the substrate unreacted. Therefore, it was decided to examine some of the strains of yeast available in our lab, which could result in the desired transformation in stereocontrolled manner. The details of our results are presented here.

2. Materials and methods

Culture. Baker's yeast (Commercial), *Candida krusei* (ATCC 14243), *Pichia farinosa* (NRRL Y110) and *Sacchromyces* sp. (soil isolate) were maintained on a medium containing potato infusion, 20%; dextrose, 2%; agar, 1.5%; and racemic 5-acetyl-3-substituted phenyl-2-isoxazoline, 0.1%. The cultures used in this study are deposited in culture collection of the institute.

Growth of organisms and preparation of cells for biotransformation. The yeast(s) were grown in medium containing yeast extract, 0.25%; glucose, 1.0%; peptone, 0.25%; KH_2PO_4, 1.0%; $(NH_4)_2HPO_4$, 2.0%; $MgSO_4 \cdot 7H_2O$, 0.025%; substrate, 0.1%, for 36 h at 28 °C. The cell suspension obtained was used as an inoculum (10%) for further cultivation under identical cultural conditions. The cells obtained after 5-sequential transfers were used as inoculum (10%) for the final cultivation. After 24 h of final growth, cells were harvested by centrifugation, washed with 10 mM phosphate buffer (pH 7.0). The washed cells (50 g) were suspended in 100 ml of reaction buffer [50 mM phosphate buffer (pH 7.0) containing $(NH_4)_2HPO_4$, 0.2%; $MgSO_4.7H_2O$, 0.025%] in a 1 L Erlenmeyer flask and incubated at 37 °C for 8 h under reciprocal agitation with an agitation speed of 50 strokes/min, prior to the addition of substrate for reduction.

Reduction. The biotransformation was initiated by the addition of solution of appropriate compound from **1a**–**b** (1 g) in DMF (2 ml) to the preincubated yeast cells. The mixture was incubated at 37 °C for 18 h under reciprocal shaking at 240 rpm. Thereafter the reaction broth was extracted with ethyl acetate (4 × 200 ml), the organic layers were combined and washed with brine (400 ml). The organic layer was dried over anhydrous sodium sulfate and concentrated under vacuo to obtain an

oily residue, which was subjected to chromatography over silica gel (230–400 mesh). Elution with hexanes:ethyl acetate (60:40, v/v) furnished the *RS*-alcohol (**3**) first followed by *SS*-alcohol (**2**) as white solids.

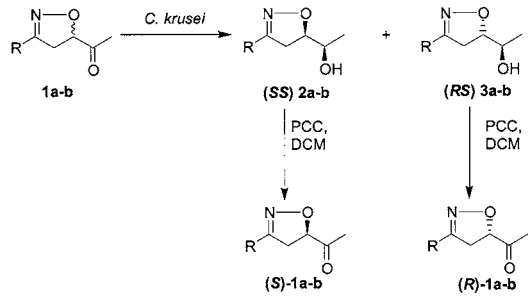
Oxidation. To the stirred solution of appropriate compound from **2** (**a**–**b**) or **3** (**a**–**b**) (0.05 mmol) in dry DCM (10 ml) was added pyridinium chlorochromate (0.075 mmol) at room temperature. The reaction was further continued for 18 h and on completion was filtered through a small band of florisil using chloroform:ethyl acetate (95:5, v/v). Evaporation of solvent under vacuo furnished the corresponding ketones as white solids.

Analysis. Melting points were determined on hot stage apparatus and are uncorrected. IR spectra were recorded on Perkin-Elmer 881 spectrophotometer. ^{1}HNMR were measured on Bruker 300/200 FTNMR while EI mass spectra were recorded on a JEOL-D-300 spectrometer. The ESMS spectra were recorded on Merck 8000 LC/ 3DQMS system through flow injections. Elemental analysis were carried out on Carlo–Erba EA1108 analyzer and $[\alpha]_d$ were recorded on Rudolph's Autopol III. The *ee* were calculated by making the MTPA ester as reported earlier [15].

3. Results and discussion

The 5-acetyl-4,5-dihydro-3-substituted phenyl-isoxazolines were obtained by 1,3-dipolar cycloadditions of a nitrile oxide on methyl vinyl ketone as described earlier [18]. The racemic 5-acetyl-isoxazolines (**1a**–**b**) were then subjected to enzymatic reduction in the presence of four different strains of yeast viz. Baker's yeast, *C. krusei*, *P. farinosa* and *Sacchromyces* sp. It was decided to use the induced resting cells of yeast available because we have observed that adaptation of the liquid-culture to the substrate by sequential transfers increase the substrate-tolerance and provides high yields of desired transformation. This is possibly due to the enhancement of the cellular pool of the desired enzyme(s). The asymmetric reduction of wide spectrum of ketones has been possible with the easy-to-use method of resting cells [19–23]. To overcome problems in using free enzymes such as denaturation, replinshment of cofactors, we also decided to use the whole cells since they offer advantage of cofactors recycling in vivo and eliminate the expensive enzyme purification. Except for the Baker's yeast, which was purchased from the market all other strains used for reactions were maintained in our Fermentation division. The *Sacchromyces* sp. that is the soil isolate is being regularly employed for the fermentation of molasses to produce alcohol in our division. It was observed that all the yeast species reduced the ketone in a stereospecific manner to the corresponding *S*-alcohol thereby affording two diastereomers as *SS*-(**2**) and *RS*-(**3**) alcohols (Scheme 1). The percent conversion and the relative ratio of the *SS*- and *RS*-alcohol are shown in Table 1.

Among all yeasts, *Sacchromyces* sp. showed maximum conversion as no ketone was recovered unreacted. Both the diastereomers of alcohol could be easily separated on silica gel using ethyl acetate:hexanes (40:60, v/v) mixture as eluent. The ratio of both diastereomers was found to be 1:1 and the optical activity of both alcohols matched the literature values suggesting that this enzyme though reduced the ketone



Scheme 1.

Table 1
Results[a] of the various strains of yeast used for reduction

Yeast strains	Substrate level (g/100 ml)	Ketone recovered (g)[b]	Total yield of recovered (g)	Conversion (%)	Relative ratio of diastereomers (syn:anti)
Baker's yeast	1	0.5615 ± 0.0662 (mixture)	0.3486 ± 0.0709 (mixture)	35	1:1
Sacchromyces sp	1	No ketone detected	0.8564 ± 0.0593 (mixture)	86	1:1
C. krusei	1	0.4175 ± 0.0425 (*R*)	0.4326 ± 0.0253 (*SS*)	44	1:0.005
P. farinosa	1	0.8129 ± 0.0275 (mixture)	0.1238 ± 0.0308 (mixture)	12	3:2

[a] Values are mean ± SD of five different experiments.
[b] Mixture here refers to diastereomeric mixture of (*R*) and (*S*)-ketones.

in an enantiospecific manner to yield only *S*-alcohol, was not diastereoselective. It was also observed that the rate of reaction varied from batch to batch of cells used, even though the cells were grown under identical conditions. This may be attributed to the fact that in growing cells, different enzymes are induced and often compete for the substrate and many reactions occur at the same time often giving mixture of products derived from different catalytic capabilities [24–27]. In contrast, the reduction with *C. krusei* was found to be diastereoselective for *S* isomer leading to the formation of alcohol (*S*,*S*)-**2** and ketone (*R*)-**1**; with only minor contamination of alcohol (*R*,*S*)-**3**. In reactions with this enzyme, no significant batch-to-batch variation was observed. We examined the baker's yeast from five different sources (five different market brands) and found that the best yield achieved was around 35% but the reaction was not stereoselective since alcohols (*S*,*S*)-**2** and (*R*,*S*)-**3** were obtained in 1:1 ratio after column chromatography. The recovered starting substrate was devoid of any optical activity confirming that the enzyme was not stereoselective. In this case it was assumed that the ability of baker's yeast to carry out reduction is dependent on the fermentative activity of the yeast cells, which may decrease as a result of gradual inactivation of enzymes and regulatory proteins during the storage

[28]. The most unsatisfactory results were obtained during the reactions with *P. farinosa* since the amount of reduction was less and also no selectivity was observed. These alcohols were further oxidized in the presence of pyridinium chlorochromate to furnish the pure (*R*)- and (*S*)-5-acetyl-3-substituted phenyl-isoxazolines.

In conclusion, we have described the effect of various yeasts strain towards the stereocontrolled reduction of 5-acetyl-3-substituted phenyl-2-isoxazolines to obtain the optically pure alcohols, which can be oxidized back to afford the enantiomerically pure isoxazolines. During our study we have found that *C. krusei* can also effect stereocontrolled reduction at very high substrate ratio with no special reaction conditions involved.

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[29] Data of compounds: (*R*)-**1a** m. p. 54–56 °C, $[\alpha]_D^{24} = -198$ ($c = 1.5$, $CHCl_3$); (*S*)-**1a** m. p. 52–53 °C, $[\alpha]_D^{24} = +197$ ($c = 1.5$, $CHCl_3$); **2a** (*1S*)-1-[(*5S*) 4,5-dihydro-3-phenyl-5-isoxazolyl] ethanol m. p. 82–84 °C, $[\alpha]_D^{24} = +165$ ($c = 2.0$, $CHCl_3$), IR (KBr): 3365 cm^{-1}, ^1H NMR (300 MHz, $CDCl_3$) δ 1.29 (d, 3H, $J = 6.6$ Hz), 2.18 (brs, lH), 3.18 (dd, lH, $J = 7.8$, 16.6 Hz), 3.40 (dd, lH, $J = 10.8$, 16.6 Hz), 3.79 (brs, lH), 4.59 (ddd, lH, $J = 4.8$, 7.5, 10.8 Hz), 7.41 (m, 3H), 7.67 (m, 2H), ^{13}C NMR (75 MHz, $CDCl_3$): δ 19.37, 37.44, 69.60, 85.14, 127.10, 129.11, 129.70, 130.60, 157.40 ppm, MS (EI): 191 (35.0), 146 (30.6), 104 (100); **3a** (*1S*)-1-[(*5R*)4,5-dihydro-3-phenyl-5-isoxazolyl] ethanol m. p. 102–104 °C, $[\alpha]_D^{24} = -145$ ($c = 2.0$, $CHCl_3$), IR (KBr): 3504 cm^{-1}, ^1H NMR (300 MHz, $CDCl_3$) δ 1.22 (d, 3H, $J = 6.6$ Hz), 2.03 (brs, lH), 3.23 (dd, lH, $J = 10.9$, 16.6 Hz), 3.40 (dd, IH, $J = 8.7$, 16.6 Hz), 4.13 (brs, IH), 4.66 (ddd, IH, $J = 5.1$, 7.8, 10.8 Hz), 7.41 (m, 3H), 7.67 (m, 2H), ^{13}C NMR (75 MHz, $CDCl_3$): δ 18.51, 34.65, 67.23, 85.57, 127.11, 129.08, 130.53, 157.16 ppm, MS (EI): 191 (37.0), 146 (30.8), 104 (100). (*R*)-**1b** m. p. 102–104 °C, $[\alpha]_D^{24} = -188$ ($c = 0.5$, $CHCl_3$); (*S*)-**1b** m. p. 94–95 °C, $[\alpha]_D^{24} = +189$ ($c = 0.5$, $CHCl_3$); **2b** (*1S*)-1-[(*5S*)4,5-dihydro-3-(4-methyl-phenyl)-5-isoxazolyl] ethanol m. p. 114–116 °C, $[\alpha]_D^{24} = +158$ ($c = 0.7$, $CHCl_3$), IR (KBr): 3384 cm^{-1}, ^1H NMR (200 MHz, $CDCl_3$) δ 1.28 (d, 3H, $J = 6.4$ Hz), 2.17 (d, 1H, $J = 5.6$ Hz), 2.37 (s, 3H), 3.14 (dd, 1H, $J = 7.5$, 16.8 Hz), 3.40 (dd, 1H, $J = 10.6$, 16.5 Hz), 3.77 (m, 1H), 4.56 (ddd, 1H, $J = 3.1$, 7.8, 11.2 Hz), 7.21 (d, 2H, $J = 8.0$ Hz), 7.55 (d, 2H, $J = 8.0$ Hz), MS (ESMS): 206.20 ($M^+ + 1$); **3b** (*1S*)-1-[(*5R*)4,5-dihydro-3-(4-methyl-phenyl)-5-isoxazolyl] ethanol m. p. 102–104 °C, $[\alpha]_D^{24} = -138$ ($c = 0.9$, $CHCl_3$), IR (KBr): 3420 cm^{-1}, ^1H NMR (200 MHz, $CDCl_3$) δ 1.22 (d, 3H, $J = 6.5$ Hz), 1.94 (d, 1H, $J = 5.5$ Hz), 2.37 (s, 3H), 3.21 (dd, 1H, $J = 10.7$, 16.6 Hz), 3.39 (dd, 1H, $J = 8.7$, 16.6 Hz), 4.13 (m, 1H), 4.64 (ddd, 1H, $J = 3.2$, 7.8, 11.1 Hz), 7.21 (m, 3H), 7.56 (m, 2H), MS (ESMSI): 206.33 ($M^+ + 1$).