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# ENANTIOSELECTIVITY IN MICROBIAL REDUCTION OF PROCHIRAL CARBONYL GROUPS: A WIDE SCREENING TOWARD *R* AND *S* ISOMERS

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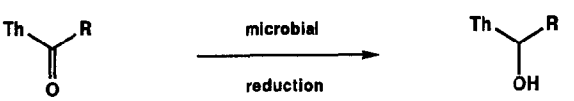
**Abstract:** More than fifty yeast and mould strains were tested in the reduction of 2-acylthiazoles **1-5**. The possibility of obtaining *R* or *S* enantiomers with high enantioselectivities from various species and strains is described.

Utilisation of biochemical systems to effect selective transformations of synthetic substrates is a useful method to provide optically pure intermediates for synthesis.[1] Microbial reduction of prochiral carbonyl groups to prepare chiral alcohols is wide spread and very efficient.[1,2,3] However, it can be difficult to obtain both enantiomers since most microorganisms give the same enantiomer that with *S*-configuration, i.e. Baker's yeast.[2] Another drawback in microbial reductions is that the optical yield is not always satisfactory and their applications, yeast reduction in particular,[4] have been hampered by lack of reproducibility.

In the present work we have systematically studied the effect of varying the structure of the substrate using a selection of yeast and mould species and strains in the reduction of prochiral carbonyl groups. 2-Acylthiazoles are selected as models since protected α-hydroxy aldehydes are useful starting material for the synthesis of biologically active compounds[5] and the thiazole ring is well-known to be synthetically equivalent to formyl group.[6]

Previous papers report the reduction of 2-acylthiazoles with Baker's yeast giving different results depending on the substrate: i) good yield and enantioselectivity (2-acetylthiazole[7] and 2-dichloroacetylthiazole[7b]); ii) poor yield and enantioselectivity (2-(acetoxy)acetylthiazole[7a]); iii) no reduction products (2-isobutyrylthiazole[7b]). The results of the reduction of the model substrates **1-5** by a large number of yeast and mould strains are summarized in the Table.




**1 - 5**

**Th** = 2-thiazolyl; **1**, R = $CH_3$; **2**, R = $CH(CH_3)_2$; **3**, R = $(CH_2)_2COOCH_3$; **4**, R = $CHCl_2$; **5**, R = $(CH_2)_8CH{=}CH_2$

The species under examination, apart from *Saccharomyces cerevisiae*, have been widely used in reduction reactions and were chosen on the basis of their recognized hydrolytic and oxido-reductive activities or on the basis of their complex role in food fermentation.[8]

**Table.** Enantioselectivity in microbial reduction of 2-acylthiazoles

| *Microorganism* [a] | 1 | | 2 | | 3 | | 4 | |
|---|---|---|---|---|---|---|---|---|
| | %[b] | ee[c] | %[b] | ee[c] | %[b] | ee[c] | %[b] | ee[c] |
| *Trichoderma viride* CBS 189.79 | 89 | 76 (*S*) | -- | -- | -- | -- | 98 | > 95 (*R*) |
| *Penicillum roqueforti* CBS 221.30 | 20 | >95 (*S*) | -- | -- | A[d] | -- | --[e] | -- |
| *Penicillum digitatum* M 161.82 | 38 | >95 (*S*) | 2 | 14 (*S*) | A[d] | -- | --[e] | -- |
| *Trichoderma sp.* | -- | -- | -- | -- | 40[f] | 10 (*S*) | 98 | 16 (*S*) |
| *Rhizopus arrhizus* | 10 | >95 (*S*) | 14 | 48 (*R*) | A[d] | -- | 9 | 30 (*S*) |
| *Fusarium sp.* | 32 | 74 (*S*) | -- | -- | A[d] | -- | --[e] | -- |
| *Alternaria sp.* DC 8 | 50 | 92 (*S*) | -- | -- | -- | -- | 98 | 0 |
| *Rhizopus nigricans* | 88 | >95 (*S*) | 61 | 90 (*R*) | -- | -- | --[e] | -- |
| *Rhizopus microsporus* | 11 | >95 (*S*) | 13 | 68 (*R*) | 28[f] | >95 (*S*) | --[e] | -- |
| *Rhizopus oryzae* CBS 372.73 | 82 | >95 (*S*) | 92 | >95 (*R*) | A[d] | -- | --[e] | -- |
| *Aspergillus niger* AN 21.181 | 47 | >95 (*S*) | -- | -- | A[d] | -- | --[e] | -- |
| *Rhizopus microsporus chinensis* CBS 346.49 | 17 | >95 (*S*) | 13 | 62 (*R*) | 89 | -- | 90 | >95 (*S*) |
| *Mucor spirescens M.A.* | 57 | >95 (*S*) | 4 | 8 (*R*) | A[d] | -- | --[e] | -- |
| *Mucor rouxianus S.Z.E.D* | 53 | >95 (*S*) | 5 | 16 (*R*) | A[d] | -- | --[e] | -- |
| *Candida utilis* CBS 621 | 78 | >95 (*S*) | -- | -- | -- | -- | -- | -- |
| *Candida steatolytica* CBS 5839 | 52 | >95 (*S*) | 78 | 86 (*R*) | -- | -- | 16 | 52 (*S*) |
| *Saccharomyces cerevisiae* RM1 (subsp. *globosus*) | 37 | 88 (*S*) | -- | -- | 139 | -- | --[e] | -- |
| *Saccharomyces cerevisiae* RM3 | 28 | >95 (*S*) | 7 | 12 (*S*) | -- | -- | --[e] | -- |
| *Saccharomyces cerevisiae* RM9 (subsp. *capensis*) | 52 | 84 (*S*) | 9 | 22 (*S*) | 99 | -- | --[e] | -- |
| *Saccharomyces cerevisiae* RM74 | 74 | >95 (*S*) | 5 | 54 (*S*) | A[d] | -- | --[e] | -- |
| *Saccharomyces cerevisiae* ML38 (subsp. *steineri*) | 37 | >95 (*S*) | 23 | 48 (*R*) | 15[f] | 6 (*R*) | --[e] | -- |
| *Saccharomyces cerevisiae* ML77 (subsp. *chevalieri*) | 98 | 72 (*S*) | 47 | 58 (*S*) | 35 | -- | --[e] | -- |
| *Saccharomyces cerevisiae* ML27 (subsp. *chevalieri*) | 98 | 84 (*S*) | 64 | 64 (*S*) | 90[f] | 40 (*R*) | --[e] | -- |
| *Saccharomyces cerevisiae* BG9 | 37 | 90 (*S*) | 61 | 80 (*S*) | 58[f] | >95 (R) | --[e] | -- |
| *Saccharomyces cerevisiae* MUT207 | 47 | >95 (*S*) | -- | -- | 5 | -- | --[e] | -- |
| *Saccharomyces cerevisiae* ML30 (subsp. *chevalieri*) | 98 | >95 (*S*) | 72 | 76 (*S*) | 99[f] | 36 (*R*) | --[e] | -- |
| *Pichia membranaefaciens* BG13 | 86 | >95 (*S*) | -- | -- | A[d] | -- | --[e] | -- |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| *Yarrowia lipolytica* | E | 45 | >95 (*S*) | -- | -- | -- | -- | 70 | 26 (*S*) |
| *Yarrowia lipolytica* | B | 74 | 80 (*S*) | -- | -- | A[d] | -- | 59 | 62 (*S*) |
| *Yarrowia lipolytica* | F | 17 | >95 (*R*) | -- | -- | A[d] | -- | 79 | 42 (*S*) |
| *Yarrowia lipolytica* | T | 69 | 86 (*S*) | -- | -- | A[d] | -- | 76 | 26 (*S*) |
| *Yarrowia lipolytica* | A | 70 | 86 (*S*) | -- | -- | A[d] | -- | --[e] | -- |
| *Yarrowia lipolytica* | Z | 9 | >95 (*S*) | -- | -- | A[d] | -- | 78 | 27 (*S*) |
| *Yarrowia lipolytica* | AB | 70 | 80 (*S*) | -- | -- | A[d] | -- | 96 | 52 (*S*) |
| *Yarrowia lipolytica* | AC | 80 | 84 (*S*) | -- | -- | A[d] | -- | --[e] | -- |
| *Yarrowia lipolytica* | BG 14 | 83 | 80 (*S*) | -- | -- | A[d] | -- | 74 | 44 (*S*) |
| *Yarrowia lipolytica* | AD | 64 | >95 (*S*) | -- | -- | -- | -- | --[e] | -- |
| *Yarrowia lipolytica* | G | 17 | >95 (*R*) | -- | -- | -- | -- | --[e] | -- |
| *Yarrowia lipolytica* | 5A | 25 | 88 (*S*) | -- | -- | A[d] | -- | 96 | 44 (*S*) |

[a] The yeast and mould cultures, except those labeled CBS, belong to DPVA collection. [b] Yields are determined by GC (on Carbowax 10% in chromosorb) at properly adjusted isothermal levels: **1**, 150° C; **2**, 160° C; **3**, 220° C; **4**, 210° C; **5**, 220° C. [c] Determined by GLC by comparison with the racemic compound; absolute configuration in parenthesis. [d] No reduction product but only the ester is hydrolyzed to the corresponding acid. [e] The yeast or mould culture is not grown. [f] Enantiomeric ratio is determined by GLC on the silylated product (hexamethyldisilazane, trimethylchlorosilane, pyridine). [g] The corresponding acid is present together with the reduction product (low yield).

A typical reduction procedure is as follows: to a yeast or mould culture (8 ml),[9] grown for 48 h in the presence of small amounts of the selected substrate (0.01 ml),[10] is added a further 0.04 ml of the substrate solution[10] and the incubation continued for a further 48 h at 25° C. The suspension is removed by centrifugation, the mixture is extracted with diethyl ether and dried over anhydrous $Na_2SO_4$. The crude reduction products are analyzed by GLC on a chiral column.[11]

In all cases 2-acetylthiazole **1** is reduced with good yields and enantioselectivity to the *S*-enantiomer[12] except with *Yarrowia lypolitica* G and F in which the *R*-enantiomer is obtained (17% yield, ee > 95%). Lower yields are obtained with **2** (R = isopropyl), however, *Rhizopus oryzae*, *Rhizopus nigricans* and *Candida steatolytica* give interesting results: the yields and the enantioselectivity are good with the prevalence of the *R*-enantiomer.[13] On the other hand, a series of *Saccharomyces cerevisiae*, i.e. ML38, ML77, ML27, BG9, and ML30, give satisfactory yields and good enantioselectivities with the prevalence of the *S*-enantiomers. With the substrate **3** (R = $(CH_2)_2COOMe$) in some cases only the hydrolysis of the ester, in others both reduction and hydrolysis are found. The most significant results are those with *Rhizopus microsporus* (28% yield, ee > 95% of the *S*-enantiomer) and *Saccharomyces cerevisiae*, i.e. BG9, (58% yield, ee > 95% of the R-isomer). Quantitative yields but lower enantiomeric excesses are obtained with *Saccharomyces cerevisiae*, i.e. ML27 and ML30 (ee 40% and 36% of the *R*-enantiomer respectively).[14] 2-Dichloroacetylthiazole **4** shows high toxicity toward most of the microorganisms whose growth is inhibited. However, excellent results are obtained with *Trichoderma viride* (98% yield, ee > 95% of the *R*-enantiomer) and *Rhizopus microsporus chinensis* (90% yield, ee > 95% of the *S*-enantiomer); surprisingly *Alternaria* give quantitative yields but no resolution.[14] Poor results are obtained with substrate **5** (R =

$(CH_2)_8CH=CH_2$): most of the the microorganisms did not effect reduction, probably because of the low solubility in the medium, except *Candida steatolytica* ( 37% yield , ee 56% of the *S*-enantiomer). Other yeast and mould strains[15] are tested but they did not reduce any of the substrates selected. On the basis of these preliminary results studies directed to the optimization of the reaction conditions are in course. The possibility of applying this information to other heterocyclic rings is also being considered.

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9. A synthetic culture medium containing for 1 l of water glucose (50 g), $(NH_4)_2SO_4$ (5 g), $KH_2PO_4$ (2 g), $CaCl_2$ (0.25 g), $MgSO_4.7H_2O$ (0.25 g), inositol (25 mg), $H_3BO_3$ (1 mg), $ZnSO_4$ (1 mg), $MnCl_2$ (1 mg), $FeCl_2$ (0.5 mg), $CuSO_4$ (0.1 mg), KI (0.1 mg), tiamine (0.3 mg), biotine (0.025 mg), calcium pantothenate (0.3 mg), pyridoxine (0.3 mg) and nicotinic acid (0.3 mg) is inoculated with a spore suspension and grown at 25° C.
10. The solution is prepared dissolving 0.4 g of the selected 2-acylthiazole in 2 ml of ethanol.
11. Enantiomer separation on Megadex 1 column (25 m X 0.32 mm) containing permethylated β-cyclodextrine in OV 1701 from Mega s.n.c.: carrier gas: helium (1 atm); temp: 100-200° C. Retention time in min: **1** (2.5° C/min) 14.4 and 14.6; **2** (2.5° C/min) 19.4 and 19.7; **3** after silylation (3° C/min) 23.5 and 23.6; **4** (2.5° C/min) 31.4 and 31.6; **5** (2.5° C/min) 48.8 and 49.3.
12. The relation beetween the absolute configuration and the retention time is established comparing the retention time of the reduction product by Baker's yeast whose *S* -configuration is confirmed converting the thiazole ring in the formyl group (*S* -lactaldehyde).
13. The absolute configuration of **2** is determined on the basis of the data obtained for **1**, assuming that if in a homologous series the *R* isomer has the lowest retention time; this a standard feature in all series.
14. The relation between the absolute configuration and the retention time is established comparing the alcohol obtained by Baker's yeast (*S* -enantiomer).
15. The other microorganisms tested are: *Botrytis*, *Debaryomyces hansenii*, *Saccharomyces cerevisiae* 692, *Rhodotaule sp.* 63 BR, *Hanseniaspora uvarum* AR 17, *Saccharomyces carlsbergiensis* 9080, *Saccharomyces cerevisiae* 635 (subsp. *cerevisiae* ), *Zygosaccharomyces bailii* ATCC 8099, *Saccharomyces cerevisiae* RM 100, *Saccharomyces cerevisiae* CO10 (subsp. *cerevisiae* ), *Saccharomyces cerevisiae* ML19 (subsp. *globosus* ), *Saccharomyces cerevisiae* ML52 (subsp. *aceti* ), and *Saccharomyces cerevisiae* ML68 (subsp. *globosus* ).