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Synthesis of Homochiral *syn*- and *anti*-α-(Hydroxyethyl)-γ-butyrolactones *via* Microbial Reduction

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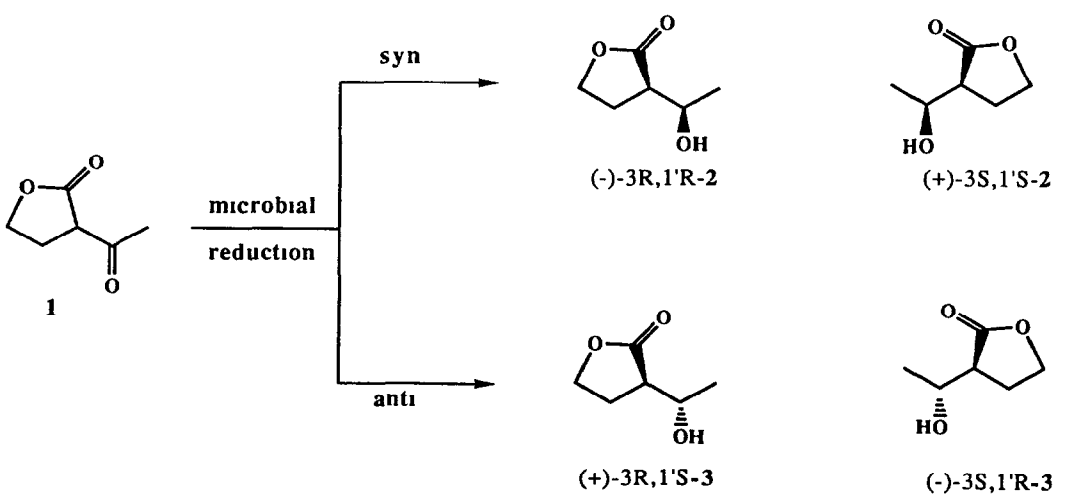
Abstract Various yeast and mould strains were tested in the reduction of α-acetyl-γ-butyrolactone *syn*-(3R,1'R)-α-(Hydroxyethyl)-γ-butyrolactone **2** and *anti*-(3R,1'S) isomer **3** were obtained enantiomerically pure Good enantiomeric excesses are described for the corresponding enantiomers

Lactone derivatives are useful intermediate in the synthesis of natural products Most of them are chiral and their physiological activity often depends on the absolute configuration [1] In this field α-acetyl-γ-butyrolactone represents a useful chiral synthon for the synthesis of triols by enantioselective reduction with Baker's yeast[2] and subsequent reductive cleavage of the lactone ring, or by initial chemical reduction and subsequent resolution of the racemic alcohols with lipase [3] In the present work we systematically studied the reduction of α-acetyl-γ-butyrolactone **1** using a selection of yeast and mould species and strains in order to obtain *syn* and *anti* isomers enantiomerically pure (Scheme)



The species under examination, apart from *Saccharomyces cerevisiae*, have been widely used in reduction reactions and were chosen both on the basis of their recognized hydrolytic and oxido-reductive activities or of their complex role in food fermentation[4] and of the results obtained in our previous studies concerning the microbial reduction of prochiral carbonyl compounds.[5] The results of the screening are summarized in Table 1

Table 1. Screening of microbial reduction of α-acetyl-γ-butyrolactone **1**

microorganism[a]	3R,1'R-2 % (ee)[b]	3S,1'S-2 % (ee)[b]	3R,1'S-3 % (ee)[b]	3S,1'R-3 % (ee)[b]
Saccharomyces cerevisiae ML77	100 (100)	—	—	—
Saccharomyces cerevisiae RM1	—	61 (44)	39 (100)	—
Saccharomyces cerevisiae RM9	100 (44)	—	—	—
Saccharomyces cerevisiae RM74	—	100 (32)	—	—
Yarrowia lipolytica PFL12A	60 (100)	—	—	—
Yarrowia lipolytica PFL9CE	100 (100)	—	—	—
Yarrowia lipolytica 5E	36 (100)	—	—	—
Mucor spinescens	60 (76)	—	26 (100)	—
Penicillum roqueforti CBS265 55	44 (90)	—	8 (28)	—
Penicillum digitatum M1618 2	80 (95)	—	11 (48)	—
Ceratocystis moniliformis CBS773 73	60 (86)	—	40 (100)	—
Rhizopus oryzae CBS285 55	51 (70)	—	33 (6)	—
Rhizopus nigricans	86 (82)	—	7 (10)	—
Fusarium	67 (81)	—	7 (8)	—
Trichoderma sp	71 (64)	—	—	29 (50)
Alternaria sp	68 (86)	—	32 (92)	—

[a]The yeast and mould cultures, except those labeled CBS, belong to the collection of Dipartimento di Protezione e Valorizzazione Agroalimentare of the University of Bologna (Italy) [b]Yields are calculated from glc analysis and enantiomeric excesses are obtained after trifluoroacetylation (trifluoroacetic anhydride, 30 min at r t)

A typical reduction procedure for the screening is as follows to a yeast or mould culture (8 ml),[6] grown for 48 h in the presence of small amount of α-acetyl-γ-butyrolactone (0 025 ml)[7], is added a further 0 075 ml of the substrate solution and the incubation continued for a further 48 h at 29 °C The suspension is removed by centrifugation, the mixture is extracted with diethyl ether and dried over anhydrous Na_2SO_4 The crude reaction products are analyzed by GLC on a chiral column [8] The stereochemical assignment of GLC peaks has been performed by a chemo-enzymatic method $NaBH_4$ reduction of the lactone **1** afforded the two diastereomeric pairs which were analyzed by ^1H NMR spectra of the 1,3-dioxane derivatives of the corresponding triols obtained by $LiAlH_4$ reduction [2] The synthesis of enantiomeric enriched (3R,1'R)-2 and (3S,1'R)-3 has been

performed by enantioselective hydrolysis of acetyl derivatives of the corresponding diastereomeric pairs, mediated by *Pseudomonas fluorescens* and *Candida cilindracea* lipases [3]

As shown in Table 1 almost all of the microorganisms gave preferentially (3R,1'R)-α-(hydroxyethyl)-γ-butyrolactone **2** in particular this compound is obtained in quantitative yield and with excellent enantiomeric excess (100%) by reduction with *Sacch cerevisiae* ML77 and *Yarrowia lipolytica* PFL9CE Among the yeasts only *Sacch cerevisiae* RM1 and RM74 afforded the 3S,1'S-2 enantiomer although with poor ee (32-44%) On the other hand the same *Sacch cerev* RM1 produced also the pure 3R,1'S-enantiomer **3** (39%, ee 100%) It is worth mentioning that only this microorganism gave the same distribution of products obtained with Baker's yeast by M Takeshita [2] We can also point out that the mould strains are less diastereoselective than yeasts giving together with 3R,1'R-**2** also 3R,1'S-**3** in some cases with excellent enantiomeric excesses (100%, i e *Mucor spirescens* and *Ceratocystis moniliformis*) Only *Trichoderma sp* afforded the 3S,1'R-enantiomer **3** (29% yield, ee 50%)

On the basis of these data some reactions were repeated on a preparative scale (culture medium 160 ml, 0 3 g of the lactone in 1 ml of ethanol) using the same methodology (preincubation, 48 h at 29 °C). The reactions were filtered through celite, a saturated solution of NaCl was added and then the mixture was extracted with ethyl acetate If only one diastereoisomer was obtained, the reaction mixture was chromatographed on silica gel with diethyl ether/petroleum ether 7 3 as eluent When both diastereoisomers were present, the separation was achieved by flash chromatography on silica gel with chloroform/methanol 50 1 as eluent The preparative data are summarized in Table 2

Table 2 Microbial reduction of the lactone **1**

microorganism	**1** %[a]	(-)-**2** %[a] (ee)	(+)-**2** % [a](ee)	(+)-**3** %[a] (ee)	(-)-**3** % [a](ee)
Saccharomyces cerevisiae ML77	30	62 (100)			
Saccharomyces cerevisiae RM1	40		25 (66)	19 (100)[b]	
Yarrowia lipolytica PFL9CE	14	69 (100)[c]			
Ceratocystis moniliformis CBS773 73	3	63 (80)		23 (90)	
Trichoderma sp	9	47 (50)			24 (50)

[a] Yields after chromatography [b] $[\alpha]_D$ = 16 8 (c 1 7, $CHCl_3$) [c] $[\alpha]_D$ = -40 6 (c 1 8, $CHCl_3$)

On a preparative scale the results of the screening are substantially confirmed The lower yields obtained with *Saccharomyces cerevisiae* ML77 and RM1 are due to the higher concentration of the lactone **1** (50% more)

From the screening we can stress that unlike Baker's yeast that reduces the carbonyl group to the S-alcohol not identifying the existing stereogenic center (3R or 3S) of the racemic lactone **1**, most of yeasts afforded the 3R,1'R-diastereomer reducing the 3R-derivative The behaviour of the mould strains is different in that they reduce the prochiral carbonyl group of the 3R-lactone **1** to both to 1'R and 1'S-diastereomers In this series only *Trichoderma* afforded the 3S,1'R-alcohol **3**

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6. 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 29° C
7. The solution is prepared dissolving 0 4 g of α-acetyl-γ-butyrolactone **1** in 4 ml of ethanol
8. Enantiomer separation on Megadex 5 column (25 m X 0 25 mm) containing dimethyl-n-pentyl-β-cyclodextrine in OV 1701 from Mega s n c carrier gas helium (0 8 atm), Temp 100-200° C (0 5° C/min) Retention time of the *syn* - and *anti* -alcohols as trifluoroacetyl derivatives (-)-3S,1'R-**3** 1 95, (+)-3R,1'S-**3** 22 49, (-)-3R,1'R-**2** 23 33, and (+)-3S, 1'S-**2** 23 97

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