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Microbial and Enzymatic Approaches to Chiral β- and γ-Nitro Alcohols

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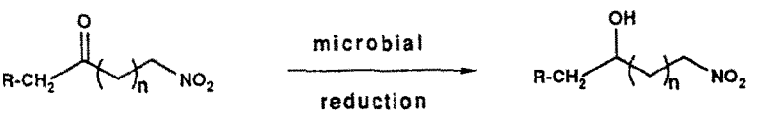
Abstract: Microbial reductions of β- and γ-nitroketones **1-3** with various yeast and mould species and strains is described. Enzyme mediated resolutions of the racemic β- and γ-nitro alcohols **4-6** and of their O-acetyl derivatives **4a-6a** are also reported.

Functionalized nitro compounds are very valuable and versatile building-blocks in organic synthesis. In particular they can be used efficiently as alkyl anion synthons through the carbon-carbon bond formation at the α-position of the nitro group. On the other hand, the easy conversion of the nitro function into carbonyl, hydrogen and other functional groups has increased the synthetic potential of the nitro alkane derivatives in the synthesis of natural products.[1] As a consequence, enantiomeric pure nitro compounds would allow the EPC syntheses[2] of important synthetic targets. Recently the reduction with Baker's yeast of 4-nitro-2-butanone and 5-nitro-2-pentanone to the corresponding S-alcohols[3] has been reported, but poor results[4] were obtained with 6-nitro-3-hexanone, an intermediate in the synthesis of chalcogran.[5]

Owing to the importance of these compounds, we described herein the enantioselectivity observed in the reduction of selected nitro ketones using a series of yeast and mould species and strains chosen on the basis of their recognized oxido-reductive activities[6] and the results obtained in the reduction of 2-acylthiazoles[7] (Scheme).



1 - 3 **4 - 6**

1, 4; R = H, n = 2; **2, 5**; R = CH_3, n = 2; **3, 6**; R = CH_3, n = 1

The yeast or mould cultures were grown in the presence of small amounts of the substrate in order to induce or activate the production of particular enzymes during the growth phase. The substrate was added to a growing culture as a concentrated solution in a relatively non toxic solvent such as ethanol and the incubation was prolonged for 48 h at 30 °C. With this procedure no microorganism grew in the presence of the compound **3** probably because of the formation of decomposition products (detected by t.l.c.) during the sterilization. However growth was achieved by adding a small amount of **3** after sterilization and reduction

products were obtained in variable yields depending on the substrate and microorganism. Lower yields were obtained if the substrate was added to the culture after the growth of the microorganisms. The results are summarized in Table I.

Table I. Microbial reduction of nitro ketones **1-3**.

Microorganism[a]	4[b]		5[b]		6[c]		6[d]	
	%[e]	ee[f]	%[e]	ee[f]	%[e]	ee[f]	%e	ee[f]
Trichoderma viride CBS 189.79	--	--	--	--	6	--	8	54(S)
Trichoderma sp.	68	52(R)	40	62(R)	83	48(R)	64	58(R)
Rhizopus arrhizus	5	16(R)	10	50(S)	--	--	--	--
Alternaria sp.	--	--	--	--	13	84(S)	45	64(S)
Rhizopus nigricans	--	--	50	36(R)	8	48(R)	--	--
Rhizopus orizae CBS 372.73	--	--	40	76(R)	47	34(R)	46	34(R)
Rhizopus mic. chinensis CBS 346.49	--	--	--	--	1.2	50(R)	--	--
Candida steatolytica CBS 5839	78	6(R)	98	48(S)	99	55(R)	95	52(R)
Sacch. cerevisiae RM9 (subsp. *capensis*)	97	92(S)	53	18(R)	30	76(S)	0.9	--
Sacch. cerevisiae RM74	82	84(S)	89	20(R)	15	76(S)	0.1	--
Sacch. cerevisiae ML38 (subsp. *steineri*)	13	100(S)	15	10(S)	--	--	--	--
Sacch. cerevisiae ML77 (subsp. *chevalieri*)	35	30(R)	20	40(R)	19	2(R)	3.5	--
Sacch. cerevisiae ML 27 (subsp. *chevalieri*)	35	8(S)	25	46(R)	21	12(S)	3	--
Sacch. cerevisiae ML31 (subsp. *chevalieri*)	40	0	20	48(R)	24	22(R)	6	8(S)
Yarrowia lipolytica Y2	80	74(S)	2	38(R)	2.8	26(R)	1.5	--
Yarrowia lipolytica Y9	44	90(S)	5	62(R)	3.5	74(R)	3	--
Yarrowia lipolytica Y6	100	60(S)	2	40(R)	3	--	3	76(R)
Yarrowia lipolytica Y4	89	68(S)	18	70(R)	6	38(R)	0.7	--
Yarrowia lipolytica Y10	65	62(S)	7	72(R)	2	50(R)	1.1	--
Yarrowia lipolytica Y3	100	64(S)	30	84(R)	2.6	48(R)	1.9	--
Yarrowia lipolytica Y5	79	72(S)	40	78(R)	0.3	--	8	84(R)

[a] The yeast and mould cultures, except those labeled CBS, belong to DPVA collection. [b] The culture media were sterilized at 120° C for 20 min in the presence of small amounts of the substrate. [c] The culture media were sterilized and then small amounts of the substrate were added. [d] The yeast and mould cultures were grown for 48 h and then the substrate was added. [e] Determined by GLC after acetylation with acetic anhydride and H_2SO_4 in catalytic amount. [f] Determined by GLC by comparison with the acetylated racemic compound; absolute configuration in parenthesis.

All yeasts (*Sacch. cerevisiae* and *Yarrowia lipolytica*) gave good yields of 5-nitro-2-pentanol 4 with the prevalence of the S-enantiomer[8] with the exception of *Sacch. cerevisiae* ML 77 which afforded the R-enantiomer (35% yield, ee = 30%). The best results are obtained with *Sacch. cerevisiae* ML 38 (13%

yield, ee = 100%) and *Sacch. cerevisiae* RM 9 (97% yield, ee = 92%). On the other hand, *Trichoderma sp.*, in contrast to the behaviour of the other mould species that did not reduce the nitro ketone **1**, gave fairly good enantiomeric excess (52%) of the R-enatiomer **4**. 6-Nitro-3-hexanone **2**, compared with compound **1**, was reduced with lower yields and with the prevalence of R-enatiomer.[8] *Yarrowia lipolytica* Y3, *Yarrowia lipolytica* Y5 and *Rhizopus orizae*, however, gave fairly good yields (30-40%) and good enantioselectivities (ee = 76-84% of the R-enantiomer). On the other hand *Candida steatolytica* produced 98% of 6-nitro-3-hexanol **5** with the prevalence of the S-enantiomer (ee = 48%). 5-Nitro-3-pentanone **3** produced in general low yields and enantioselectivities. Good results were obtained with *Trichoderma sp.* and *Candida steatolytica* (83% and 99% yields) with the prevalence of the R-enantiomer[9] (ee = 48% and 55% respectively). However, *Alternaria sp.*, *Sacch. cerevisisae* RM 9 and RM 74 afforded the prevalence of the S-enantiomer (ee = 84% , 76% and 76% respectively).

Though these results are generally satisfactory, particularly with regard to compounds **2** and **3**, we also examined the possibility of obtaining chiral nitro alcohols **4-6** *via* enzymatic acetylation of the racemate (Table II) or enzymatic hydrolysis of the racemic O-acetyl derivatives **4a-6a** (Table III).

Table II. Enzymatic resolution of the racemic nitro alcohols **4-6** *via* acetylation.

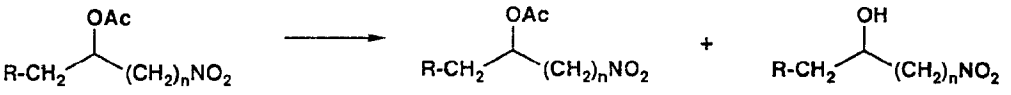
4 - 6 (R) - 4 a - 6 a

nitro alcohol	enzyme	nitro O-acetyl %	ee (abs.conf.)	time (h)
4	PPL	**4a** (38)	92 (R)	45
5	PPL	**5a** (30)	80 (R)	45
5	LAPP	**5a** (35)	10 (R)	20
5	LAPH	**5a** (15)	40 (R)	145
6	PPL	**6a** (30)	46 (R)	21

For 5-nitro-2-pentanol **4** enzymatic acetylation with a suspension of PPL in t-butyl methyl ether with vinyl acetate at 26° C. gave 38% of the R-enantiomer (ee = 92%). However worse results were obtained under the same conditions with **5** and **6** (ee = 80% and 46% of the R-enantiomer respectively) even when utilizing other enzymes (LAPP and LAPH). On the other hand, the enzymatic hydrolysis of the racemic O-acetyl derivatives **4a** with a suspension of CCL in phosphate buffer (pH = 8) and isopropanol afforded the **R-4a** with high enantioselectivity (ee = 100%). However, the hydrolysis of the racemic **5a** with CCL in the same conditions gave, after 27 h, the **R-5a** (40% yield, ee = 100%) and the S-alcohol **5** (60% yield, ee = 65%). Finally, the hydrolysis of the O-acetyl derivative **6a** produced the **S-6a** (10%, ee = 100%) with a suspension of LAPH in phosphate buffer (pH = 7) and methanol and the **R-6a** (24%, ee = 98%)

with CCL. In conclusion, this double approach (microbial and/or enzymatic) allows the EPC synthesis of these nitro alcohols.

Table III. Enzymatic resolution of the racemic O-acetyl derivatives **4a-6a** *via* hydrolysis.



4a-6a

nitro O-acetyl	enzyme	% hydrolysis	time(h)	nitro O-acetyl %	nitro O-acetyl ee (abs.conf.)
4a	LAPP[a]	74	2	**4a** (26)	80 (R)
4a	CCL[b]	64	46	**4a** (36)	100 (R)
5a	LAPP[a]	77	16	**5a** (23)	95 (R)
5a	CCL[b]	60	30	**5a** (40)	100 (R)
6a	LAPH[c]	90	18	**6a** (10)	100 (S)
6a	LAPP[d]	89	5	**6a** (11)	84 (S)
6a	CCL[b]	76	52	**6a** (24)	98 (R)

[a] The reactions are carried out in phosphate buffer (pH = 8) and t-butyl methyl ether at 28° C. [b] Reactions in phosphate buffer (pH = 8) and isopropanol at 20°C. [c] Reactions in phosphate buffer (pH = 7) and methanol at 25° C. [d] Reactions in phosphate buffer (pH = 7) and methanol at 0° C.

Experimental

^{1}H NMR spectra were obtained on 80 MHz WP80 Bruker and on 300 MHz Gemini 300 Varian spectrometers. Chemical shifts were given in parts per million from Me_4Si as internal standard. IR spectra were recorded on a Perkin Elmer Model 297 grating spectrometer. Elemental analyses were performed on a 1106 Microanalyzer (Carlo Erba). Optical rotations were measured on a Perkin Elmer Model 241 polarimeter. Gas chromatographic analyses were performed on a Carlo Erba HRGC 5160 Mega series. PPL = porcine pancreatic lipase, LAPP = liver acetone powder pig and LAPH = liver acetone powder horse are commercially available from Sigma. 5-Nitro-2-pentanone (**1**)[10], 6-nitro-3-hexanone (**2**)[5] and 5-nitro-3-pentanone (**3**)[11] are prepared according the literature procedure.

Microbial reduction of nitro ketones 1-3. General procedure. A small amount of the selected substrate **1-3** (0.01 ml)[12] is added to a culture medium (8 ml)[13] and then the solution is sterilized at 120° C for 20 min. A spore suspension is inoculated and grown at 25° C for 48 h. A further 0.04 ml of the substrate solution[12] is added and the incubation continued for 48 h at 25° C. The suspension is removed by centrifugation, the mixture is extracted with diethyl ether and dried over Na_2SO_4. After removing the solvent, the enantiomeric excesses of the crude reduction products are evaluated by acetylation with acetic anhydride and H_2SO_4 in catalytic amount and subsequent analysis by GLC on chiral column[14] (See Table I). Chromatography of the reaction mixture (silica gel, petroleum ether-diethyl ether 7 : 3) give the corresponding nitro alcohols **4-6**.

(+)-(S)-5-Nitro-2-pentanol (4) (ee = 100%)[15]: bp 73 °C/0.8 mmHg[10]; $[\alpha]_D$ = 17 (c 2.0, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 1. 17 (d, 3 H, *J* = 6.2 Hz), 1.47 (m, 2 H), 1.95-2.2 (m, 3 H), 3.8 (m, 1 H), 4.4 (t, 2 H, *J* = 7.2 Hz); ^{13}C NMR (75.5 Hz, $CDCl_3$) δ 23.15, 23.37, 34.91, 66.90, 75.50.
(+)-(S)-6-Nitro-3-hexanol (5) (ee = 65%)[16]: oil; $[\alpha]_D$ = 9.3 (c 3.2, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 0.91 (t, 3 H, *J* = 7.3 Hz), 1.36-1.60 (m, 5 H), 1.98-2.22 (m, 2 H), 3.53 (m, 1 H), 4.4 (t, 2 H, *J* = 7.1 Hz).
(-)-(R)-5-Nitro-3-pentanol (6) (ee = 98%)[17]: oil; $[\alpha]_D$ = -31.5 (c 0 65, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 0.93 (t, 3 H, *J* = 7 Hz), 1.5 (m, 2 H), 1.82 (br s, 1 H), 1.96 (m, 1 H), 2.21 (m, 1 H), 3.6 (br s, 1 H), 4.53 (m, 2 H).

Enzymatic acetylation of the racemic nitro alcohols 4 -6. General procedure. To a solution of the selected racemic alcohol (0.4 mmol) in anhydrous t-butyl methyl ether (5 mL) vinyl acetate (1.2 mmol) and the proper enzymes (6 mass equiv) were added. The suspension was vigorously stirred at 26 °C for the appropriate time. Aliquots were withdrawn periodically and their GLC chromatogram on chiral column were obtained. The results are listed in Table II. Chromatography of the reaction mixture (silica gel, petroleum ether-diethyl ether 1:1) gave the corresponding O-acetyl derivative **4a-6a**.
(-)-(R)-5-Nitro-2-O-acetylpentanol (**4a**) (ee = 92%): oil; $[\alpha]_D$ = -4 2 (c 10.0, $CHCl_3$); IR (neat) 1725, 1550 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.19 (d, 3 H, *J* = 7 Hz), 1.58 (m, 2 H), 1.90-2.10 (m, 2 H), 2.0 (s, 3 H), 3.35 (t, 2 H, *J* = 6.5 Hz), 4.90 (m, 1 H).
(+)-(R)-6-Nitro-3-O-acetylhexanol (**5a**)[18] (ee = 100%); oil; $[\alpha]_D$ = 4.5 (c 4.6, $CHCl_3$); IR (neat) 1720, 1550 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.85 (t, 3 H, *J* = 7 Hz), 1.38-1.62 (m, 4 H), 1.92-218 (m, 2 H), 2.02 (s, 3 H), 4.35 (t, 2 H, *J* = 6.5 Hz), 4.80 (m, 1 H).
(-)-(R)-5-Nitro-3-O-acetylpentanol (**6a**)[18] (ee = 98%): oil; $[\alpha]_D$ = -1.5 (c 2.2, $CHCl_3$); IR (neat) 1720, 1540 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.91 (t, 3 H, *J* = 7.1 Hz), 1.52-1.70 (m, 2 H), 2.04 (s, 3 H), 2.15-2.40 (m, 2 H), 4.41 (t, 2 H, *J* = 7.3 Hz), 4.9 (m, 1 H).

Enzymatic hydrolysis of the racemic O-acetyl derivative 4a-6a with CCL. General procedure. To a phosphate buffer solution (5 mL, pH = 8) CCL (0.3 g) was added under vigorous stirring . After 10 min a solution of the proper O-acetyl derivative (30 mg) in isopropanol (0.5 mL) was added. Stirring was continued for the appropriate time at 20 °C. Aliquots were withdrawn periodically and their GLC chromatogram on chiral column were obtained. The data are listed in Table III.

Enzymatic hydrolysis of the racemic 4a-6a with LAPP. General procedure. The reactions were carried out as above for CCL in phosphate buffer (5 mL, pH = 8) and LAPP (0.15 g). The racemic O-acetyl derivative (30 mg) dissolved in t-butyl methyl ether (0.5 mL) was added at 28 °C. For the compound **6a** a phosphate buffer at pH = 7 was used and the O-acetyl derivative in methanol (1 mL) was added at 0 °C.

Enzymatic hydrolysis of the racemic 6a with LAPH. The reaction is carried out as above for LAPP in phosphate buffer at pH = 7 and methanol as solvent at 25 °C (see Table III).

Acknowledgments

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8. The relation between the absolute configuration and the retention time was established comparing the retention time of the product from reduction by Baker's yeast whose S-configuration has been confirmed (ref 10).
9. The absolute configuration of **6** is determined on the basis of the data obtained from reduction with Baker's yeast (42% yield and ee = 60% of the S-enantiomer).
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12. The solution is prepared dissolving 0.4 g of the selected nitro ketone in 2 ml of ethanol.
13. The synthetic culture medium is prepared adding to 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).
14. 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 (2° C / min). Retention time in min after acetylation: **4**, 17.33 and 18.79; **5**, 20.85 and 21.40; **6**, 15.91 and 16.62.
15. Spectroscopic data of **S-4** are obtained from the preparative microbial reduction of **1** with *Saccharomyces cerevisiae* ML 38.
16. Spectroscopic data of **S-5** are obtained from the enzymatic hydrolysis of the racemic **5a** with CCL.
17. Spectroscopic data of **R-6** are obtained from the enzymatic hydrolysis of racemic **6a** with CCL that gave **R-6a** in 24% yield (ee = 98%). Subsequent hydrolysis of **R-6a** with MeOH/MeONa afforded **R-6** (100% yield, ee = 98% by GLC).
18. Spectroscopic data of **R-5a** and **R-6a** are obtained from the enzymatic hydrolysis of the corresponding racemic **5a** and **6a**.