Pergamon

Tetrahedron: *Asymmetry* 9 (1998) 1389–1394

TETRAHEDRON: *ASYMMETRY*

Microbial biotransformations in water/organic solvent system. Enantioselective reduction of aromatic β- and γ-nitroketones

Francesco Molinari,[a] Ernesto G. Occhiato,[b] Fabrizio Aragozzini [a] and Antonio Guarna [b,*]

[a] *Dipartimento di Scienze e Tecnologie Alimentari e Microbiologiche, Sezione Microbiologia Industriale, Università degli Studi di Milano, via Celoria 2, I-20133 Milano, Italy*

[b] *Dipartimento di Chimica Organica 'U. Schiff' and Centro dei Composti Eterociclici C.N.R., Università di Firenze, Via G. Capponi 9, I-50121 Firenze, Italy*

Received 9 February 1998; accepted 3 March 1998

Abstract

The production of single enantiomers of γ- and β-nitroalcohols by microbial bioreduction has been studied. A restricted screening among 14 yeasts was performed using 1-phenyl-4-nitro-1-butanone **1** as substrate. *Pichia minuta* (CBS 1708) and *Pichia etchellsii* (CBS 2011) gave the highest enantiopreference for (*S*)-alcohol **3** formation (e.e. 85% and 80%, respectively), while *Kluyveromyces marxianus* (CBS 397) was the only strain able to preferentially furnish the (*R*) enantiomer (e.e. 70%). These three microorganisms, along with baker's yeast, were then employed in reactions performed in water/organic solvent systems using different solvents (hexane, benzene and dibutyl ether) affording alcohol **3** in high enantiomeric excesses (>95–97%). These strains were also employed to reduce 1-phenyl-3-nitro-1-propanone **2**, maintaning the same stereobias observed with γ-nitroketone **1** and showing high enantioselectivity in both simple aqueous (>85–97%) and biphasic media (>97%).

1. Introduction

Enantiopure nitroalcohols are useful building blocks in asymmetric synthesis because they can be easily converted into many other chiral compounds through the formation of new C–C bonds at the position α to the nitro group.[1] Furthermore, nitro and hydroxy groups can be transformed into other functionalities.[1] Optically active secondary nitroalcohols have been obtained by microbial reduction of nitroketones using baker's yeast,[2–10] providing preferentially the corresponding (*S*)-nitroalcohols in accordance with Prelog's rule.[11]

Recently we have reported on the baker's yeast (*Saccharomyces cerevisiae*, type II, Sigma) reduction of aromatic γ-nitroketones which, with respect to the reduction of aliphatic γ-nitroketones performed under

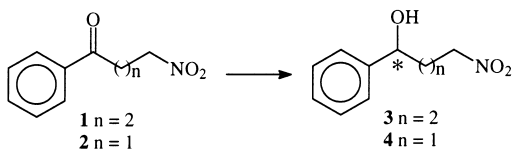
* Corresponding author. E-mail: guarna@chimorg.unifi.it

0957-4166/98/$19.00
PII: S0957-4166(98)00096-2

the same conditions, provided low yields of nitroalcohols and, in most cases, incomplete enantioselectivity, after a 7 day reaction time.[12] These results could be due to either the activity of a single enzyme with low enantiodiscrimination or the presence of various dehydrogenases which, having different selectivity, would catalyze unwanted redox equilibria causing racemization of the formed product.

Since it is known that the use of organic co-solvents in microbial reductions considerably reduces the rate of undesired reactions, particularly racemization processes,[13,14] we decided to evaluate if baker's yeast and other microorganisms could be used to catalyze the bioreduction of aromatic nitroketones in water/organic solvent systems in order to produce single enantiomers of γ- and β-nitroalcohols with high enantiomeric excess. Moreover, since the production of both the enantiomers is an important synthetic goal, we also tried to identify microorganisms able to produce nitroalcohols having an (*R*) absolute configuration.

With this in mind, 14 microorganisms[14,15] were chosen for a first screening carried out in an aqueous medium to evaluate the reduction of aromatic γ-nitroketone **1** (Scheme 1). Then, the most promising strains, along with baker's yeast, were used for the reduction of the same substrate in water/organic solvent systems and also employed for the bioreduction of β-nitroketone **2** (Scheme 1). The reduction of the latter compound in particular affords a precursor which could be easily transformed into chiral aminoalcohols which are potential biologically active compounds.[16]



Scheme 1.

2. Results and discussion

The results of the bioreduction screening on **1** in an aqueous medium, carried out in presence of glucose or ethanol as co-substrates, are reported in Table 1.

As in the reduction carried out with baker's yeast (results summarized in entry 15),[12] all the tested strains afforded the (*S*)-nitroalcohol, with the exception of *K. marxianus* (CBS 397) (entry 10) which provided the nitroalcohol with absolute (*R*) configuration in 50–70% e.e. Among the screened strains, *P. minuta* (entry 9) and *P. etchellsii*[17] (entry 12) furnished nitroalcohol **3** with enantiomeric excesses (80–85%) comparable or even superior to that obtained by using baker's yeast. All the other microorganisms failed to give better results, and in some cases (entries 13 and 14) almost racemic products were obtained. It is noteworthy that the reduction rates with all the screened strains were much higher than that observed with baker's yeast, with conversions to nitroalcohol **3** being almost complete after 24 hours. The use of different co-substrates such as ethanol or glucose, which could affect the stereochemical outcome of the bioreductions,[14] did not determine dramatic differences in the enantiomeric excesses (and molar conversions) with any of the microorganisms in these experiments, perhaps with the exception of *C. boidinii* (entry 3).

The two microorganisms which furnished the (*S*)-alcohol with the highest enantiomeric excess (*P. minuta* and *P. etchellsii*), the strain able to give the (*R*) enantiomer (*K. marxianus*), and *S. cerevisiae* type II were at this point used for the reduction of **1** in water/organic solvent systems, using different organic co-solvents as reported in Table 2. The four yeasts maintained the stereobias observed in water, but the enantiomeric excesses were much higher, up to 97%, using hexane, benzene or dibutyl ether

Table 1
Microbial reduction of **1** to alcohol **3** in water[a]

entry	microorganism	co-substrate					
		glucose[b]			ethanol[c]		
		m.c. (%)[d]	conf.[e]	e.e. (%)[e]	m.c. (%)[d]	conf.[e]	e.e. (%)[e]
1	*Candida boidinii* CBS 2428[f]	100	*S*	70	100	*S*	65
2	*Candida boidinii* CBS 3092	100	*S*	40	100	*S*	46
3	*Candida boidinii* CBS 6035	100	*S*	10	100	*S*	50
4	*Candida utilis* CBS 621	90	*S*	55	90	*S*	60
5	*Candida utilis* MIM	80	*S*	70	80	*S*	45
6	*Geotrichum candidum* MIM	90	*S*	40	90	*S*	50
7	*Hansenula anomala*	80	*S*	65	80	*S*	70
8	*Hansenula glucozyma*	90	*S*	40	90	*S*	60
9	*Pichia minuta CBS 1708*	90	*S*	85	90	*S*	85
10	*Kluyveromyces marxianus CBS 397*	80	*R*	50	80	*R*	70
11	*Kluyveromyces marxianus CBS 1533*	100	*S*	40	100	*S*	50
12	*Pichia etchellsii* CBS 2011	90	*S*	80	100	*S*	75
13	*Pichia fermentans* DBVPG 2770	50	-	0	50	*S*	10
14	*Pichia pastoris* CBS 704	80	*S*	10	80	*S*	10
15	*Saccharomyces cerevisiae* type II[g]	89[g]	*S*	78	-	-	-

[a]Reductions carried out with fresh cells (50g/L) resuspended in 0.1 M phosphate buffer (pH 6.0) at 28°C in a 10 mL screw capped test tube with a final reaction volume of 5 mL; the concentration of substrate **1** was 1.5 mg/mL. [b]The concentration of glucose was 50 mg/mL. Substrate dissolved in DMSO (50mg/mL) and added to the cell suspension. [c]Substrate dissolved in ethanol (50 mg/mL) and added to the cell suspension. [d]Molar conversion (m.c.) determined after 24 h by GC on the crude mixture. [e]Absolute configurations and e.e. determined by chiral phase GC of the Mosher's ester derivatives. [f]Abbreviations CBS, Centraal Bureau voor Schimmelcultures, Baarn, Holland; DBVPG, Dipartimento di Biologia Vegetale Perugia, Italia; MIM, Microbiologia Industriale MIlano, Italia. [g]Performed as reported with yeast from Sigma.[12] Conversion after 7 days.

Table 2
Microbial reduction of **1** to alcohol **3** in water/organic solvent systems[a]

substrate	co-solvent	*S. cerevisiae* type II		*P. minuta*		*K marxianus*		*P. etchellsii*	
		config.[b]	e.e. (%)[b]	config.[b]	e.e. (%)[b]	config.[b]	e.e. (%)[b]	config.[b]	e.e. (%)[b]
1	benzene	*S*	95	*S*	97	*R*	95	*S*	97
1	hexane	*S*	94	*S*	96	*R*	95	*S*	90
1	dibutyl ether	-	-	*S*	97	*R*	95	*S*	97

[a]Reductions carried out with fresh cells (50 g/L), with the exception of *S. cerevisiae*, suspended in a 0.1 M phosphate buffer (pH 6.0) and co-solvent mixture (1.5 : 3.5 ratio) at 28°C, in a 10 mL screw capped test tube with a final reaction volume of 5 mL; the concentration of substrate **1** was 1.5 mg/mL. Only ethanol was used as a co-substrate. Molar conversions were about 80% in all cases after 48 h, with the exception of that with *S. cerevisiae* (< 20% after 48 h). [b]Absolute configuration and e.e. determined by chiral phase GC of the Mosher's ester derivatives.

as co-solvents. In particular, (*R*)-nitroalcohol (+)-**3** was obtained with high enantiomeric purity with *K. marxianus*. The conversion rates were to some extent lower than those observed in the aqueous medium and the reductions were stopped after 48 hours (when the conversions were about 80%). The reduction carried out with baker's yeast was very slow, having a conversion lower than 20% after 48 hours. However, despite the low reduction rates observed with *S. cerevisiae*, the high enantiomeric excesses obtained make this yeast worthy of use for the reduction of aromatic nitroketone **1** in the presence of an organic solvent.

The reduction of β-nitroketone **2** was first performed using baker's yeast as reported for compound **1**.[12] The (*S*)-alcohol **4**[18] was obtained in 85% e.e. (Table 3, entry 4) and the reduction was complete after 3 days. Also *P. minuta*, *K. marxianus* and *P. etchellsii* were employed in the reduction in water of the nitroketone **2** (Table 3, entries 1–3) displaying, under the same conditions, higher enantioselectivity

Table 3
Microbial reduction of **2** to alcohol **4**[a]

entry	Microorganism	m.c. (%)[b]		e.e. (%)[c]		configuration.[c]	
		water	water/co-solvent[d]	water	water/co-solvent[d]	water	water/co-solvent[d]
1	*P. minuta*	85	80	> 97	> 97	*S*	*S*
2	*K. marxianus*	70	80	> 97	> 97	*R*	*R*
3	*P. etchellsii*	75	80	85	> 97	*S*	*S*
4	*S. cerevisiae* type II	100[e]	30[e]	85	> 97	*S*	*S*

[a]Reductions carried out with fresh cells (50 g/L), with the exception of baker's yeast, resuspended in 0.1 M phosphate buffer (pH 6.0) containing 50 mg/mL of ethanol at 28°C, or with fresh cells (50 g/L) suspended in a 0.1 M phosphate buffer (pH 6.0) and co-solvent mixture (1.5 : 3.5 ratio) at 28°C, in a 10 mL screw capped test tube with a final reaction volume of 5 mL; the concentration of substrate **2** was 1.5 mg/mL. [b]Molar conversions (m.c.) determined by GC after 36 hours on the crude mixture. [c]Absolute configuration and e.e. determined by chiral phase GC of the Mosher's ester derivatives. [d]The results obtained using hexane as a co-solvent are reported. [e]Determined after 72 h by GC.

(e.e. >97%) than in the reduction of γ-nitroketone **1**. The greater differentiation between the *large* (the phenyl ring) and the *small* (the nitroalkyl chain) groups in **2**, due to the reduced length of the nitroalkyl chain, could explain, according to Prelog's rule, the higher enantioselectivity in the reduction of the β-nitroketone.[12]

The reduction of **2** was also carried out in the same two-liquid phase systems used for the reduction of **1**. Virtually enantiopure alcohol **4** (>97% e.e.) was obtained with the four microorganisms, with conversions similar to those observed in the previous experiments. It is noteworthy that *K. marxianus* maintained the same stereobias observed in the reduction of **1**, providing the (*R*)-alcohol both in water and in water/organic solvent systems.

The extension of *P. minuta*, *P. etchellsii* and *K. marxianus* mediated reductions of **1** and **2** to a possible synthetic use was also checked. In particular, no significant differences from the results obtained in the above-described screenings were observed when performing the reduction on a mmol scale, and enantiopure (+)-**3** and (+)-**4** and their corresponding enantiomers were isolated and characterized.

In conclusion, this work demonstrates that the use of readily available yeasts in water and/or in non-conventional media allows for the production of different chiral γ- and β-nitroalcohols with high enantiomeric excess. Since the strains used in this work are suitable to be applied to a large scale synthesis of nitroalcohols, their use appears to be a general and useful method to provide enantiopure chiral precursors for asymmetric synthesis.

3. Experimental

3.1. Material and methods

Enantiomeric excesses were determined by GC analysis of the Mosher's esters[19] obtained by reaction of **3** and **4** with (*S*)-(+)-α-methoxy-α-trifluoromethylphenylacetyl chloride (MPTA-Cl) using a chiral capillary column (diameter 0.25 mm, length 25 m thickness 0.25 μ, DMePeBeta-CDX, Mega, Legnano, Italy). The absolute configuration of **3** was determined by GC analysis of its Mosher's ester in comparison with that of (*S*)-(−)-**3** obtained as reported.[12] The same method was used for the determination of the absolute configuration of **4**, using for comparison (*S*)-(−)-**4** obtained by baker's yeast reduction. *Saccaromyces cerevisiae* type II was purchased from Sigma.

3.2. Microorganisms, media and culture conditions

All the yeasts were grown at 27°C on malt broth at pH 6.0 for 36–48 h on a reciprocating shaker (100 strokes/min). Fresh cells from submerged cultures were centrifuged and washed with 0.1 M phosphate buffer (pH 6.0). Washed cells were directly used for biotransformations.

3.3. Biotransformation conditions

The reduction of **1** using *P. etchellsii* is reported as an example. *Without co-solvent.* A solution of nitroketone (75 mg) in ethanol (1.5 mL) was added to a stirred suspension (50 mL) of fresh cells of *P. etchellsii* (2.5 g) resuspended in 0.1 M phosphate buffer (pH 6.0) at 28°C. After 48 h the conversion was about 80%. Sodium chloride was added and the suspension extracted with diethyl ether. The organic layer was dried over sodium sulphate, filtered and concentrated to give crude **3**. The purification was carried out as already described to afford pure (*S*)-(−)-1-phenyl-4-nitro-1-butanol [(−)-**3**] (57 mg) in 75% yield and 70% e.e.[12] *With organic co-solvent.* A solution of nitroketone (75 mg) in ethanol (1.5 mL) was added to a suspension of fresh cells *P. etchellsii* (2.5 g) resuspended in a mixture of 0.1 M phosphate buffer (15 mL, pH 6.0) and organic solvent (35 mL). The mixture was stirred at 28°C for 48 h. Usual work-up and purification gave pure (*S*)-(−)-1-phenyl-4-nitro-1-butanol [(−)-**3**] (56 mg) in 75% yield and 97% e.e.[12] $[\alpha]_D^{25}$ −50.6 (c 0.52, $CHCl_3$).

3.4. (R)-(+)-1-Phenyl-4-nitro-1-butanol (+)-3

Prepared as described above using *K. marxianus* in a phosphate buffer/hexane two-liquid phase system. Starting from 75 mg of nitroketone **1**, alcohol (+)-**3** (57 mg) was obtained in 75% yield and 95% e.e. $[\alpha]_D^{25}$ +49.6 (c 0.50, $CHCl_3$). Spectroscopic and analytical data are identical to those reported for (−)-**3**.[12]

3.5. (S)-(−)-1-Phenyl-3-nitro-1-propanol (−)-4

Prepared as described above using *P. minuta* without co-solvent. Starting from 75 mg of nitroketone **2** enantiopure alcohol (*S*)-(−)-**4** (39 mg) was obtained in 52% yield after usual work-up followed by chromatography (eluant Et_2O, R_f 0.71) and bulb to bulb distillation (250°C, 5×10^{-1} mbar). Oil. $[\alpha]_D^{25}$ −32.3 (c 0.92, $CHCl_3$); e.e. >97%; ^{1}H NMR ($CDCl_3$) δ: 7.37–7.29 (m, 5H), 4.83 (dd, *J*=7.9, 5.5 Hz, 1H), 4.68–4.37 (m, 2H), 2.44–2.33 (m, 2H); ^{13}C NMR ($CDCl_3$) δ: 143.5 (s, 1C), 129.5 (d, 2C), 128.9 (d, 2C), 126.2 (d, 1C), 72.9 (d, 1C), 71.8 (t, 1C), 36.7 (t, 1C); MS *m/z* (%) 133 (13), 107 (81), 105 (100), 70 (70), 77 (49); IR (neat) 3606, 1551, 1363 cm^{-1}. Elemental anal. calcd for $C_9H_{11}NO_3$: C, 59.66; H, 6.12; N, 7.73. Found C, 59.34; H, 6.00; N, 7.51.

3.6. (R)-(+)-1-Phenyl-3-nitro-1-propanol (+)-4

Obtained as reported above for the reduction of **1** with *K. marxianus* without co-solvent. Starting from 75 mg of nitroketone **2**, alcohol (+)-**4** (42 mg) was obtained in 55% yield and >97% e.e. $[\alpha]_D^{25}$ +32.5 (*c* 0.83, $CHCl_3$). Spectroscopic and analytical data are identical to those reported for (−)-**4**.

Acknowledgements

We thank the Ministry of University and Scientific and Technological Research (MURST) and CNR (Italy) for financial support. E.G.O. thanks Università di Firenze for a two-year post-doctoral fellowship.

References

1. *Nitro Compounds; Recent Advances in Synthesis and Chemistry*, Edited by Feuer, H. and Nielsen, A. T. VCH Publishers, New York, 1990.
2. Hafner, T.; Reissig, H.-U. *Liebigs Ann. Chem*. **1989**, 937.
3. Nakamura, K.; Inoue, Y.; Shibahara, J.; Oka, S.; Ohno, A. *Tetrahedron Lett*. **1988**, *29*, 4769.
4. Nakamura, K.; Kitayama, T.; Inoue, Y.; Ohno, A. *Bull. Chem. Soc. Jpn* **1990**, *63*, 91.
5. Nakamura, K.; Takebe, Y.; Kitayama, T.; Ohno, A. *Tetrahedron Lett*. **1991**, *32*, 4941.
6. Fantin, G.; Fogagnolo, M.; Guerzoni, E. M.; Marotta, E.; Medici, A.; Pedrini, P. *Tetrahedron: Asymmetry* **1992**, *3*, 947.
7. Kitayama, T. *Tetrahedron* **1996**, *52*, 6139.
8. Occhiato, E. G. Guarna, A.; Spinetti, L. M. *Tetrahedron* **1993**, *49*, 10629.
9. Occhiato, E. G.; Guarna, A.; De Sarlo, F.; Scarpi, D. *Tetrahedron: Asymmetry* **1995**, *6*, 2971.
10. Forzato, C.; Nitti, P.; Pitacco, G.; Valentin, E. *Tetrahedron: Asymmetry* **1997**, *8*, 1811.
11. Prelog, V. *Pure Appl. Chem*. **1964**, *9*, 119.
12. Guarna, A.; Occhiato, E. G.; Spinetti, L. M.; Vallecchi, M. E.; Scarpi, D. *Tetrahedron* **1995**, *51*, 1775.
13. *Selective Biocatalysis. A Synthetic Approach*, Edited by Poppe, L. and Novak, L. VCH Publishers, New York, 1992.
14. Molinari, F., Bertolini, C., Aragozzini, F. *Biocatalysis and Biotransformation* **1997**, *16*, 87.
15. Ferraboschi, P.; Santaniello, E.; Tingoli, M.; Aragozzini, F.; Molinari, F. *Tetrahedron: Asymmetry* **1993**, *4*, 1931.
16. Nogrady, T. *Medicinal Chemistry. A Biochemical Approach*. Chapter 4. Oxford University Press, New York, 1985.
17. In the reduction of aromatic nitroketones *P. etchellsii* displayed a behaviour opposite to that observed in the reduction of a series of aliphatic methyl ketones, where anti-Prelog (*R*) secondary alcohols (70–85% e.e.) were always obtained.[14]
18. The determination of the absolute configuration of alcohols (−)- and (+)-**4** was possible by NMR analysis of their Mosher's ester derivatives as reported in Ref. 12.
19. (a) Dale, J. A.; Dull, D. L.; Mosher, H. S. *J. Org. Chem*. **1969**, *34*, 2543. (b) Dale, J. A.; Mosher, H. S. *J. Am. Chem. Soc*. **1973**, *95*, 512.