Stereochemical Control in Bakers' Yeast Redox Biotransformations of Aryl Methyl Ketones and Carbinols

Giancarlo Fantin,† Marco Fogagnolo,†
M. Elisabetta Guerzoni,‡ Alessandro Medici,*,†
Paola Pedrini,† and Silvia Poli†

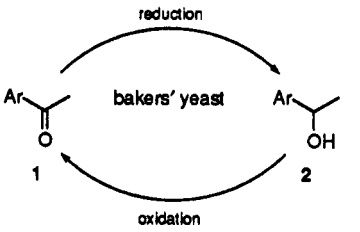
Dipartimento di Chimica, Università di Ferrara, Via Borsari 46, I-44100 Ferrara, Italy, and Dipartimento di Protezione e Valorizzazione Agroalimentare (DPVA), Sezione Chimica e Tecnologia degli Alimenti, Università di Bologna, Via S. Giacomo 7, I-40136 Bologna, Italy

Received July 29, 1993
(Revised Manuscript Received November 9, 1993)

The development of methods for the synthesis of optically active compounds has become one of the most important goals in the fields of organic chemistry; in particular, the transformations mediated by bakers' yeast, *Saccharomyces cerevisiae*, have been used for asymmetric carbonyl reduction.[1] The major problem in the use of bakers' yeast is the stereoselectivity and its control. Since most of the enzymes active in bakers' yeast will display their properties under similar conditions, it is widely reported that is the nature of the substrate which drives the reaction in one direction or the other.[1] On the other hand, it is well known that other parameters affect the course of biotransformations: pH,[2] the nature of nutrients,[3,4] concentration of the substrate,[5] cell immobilization,[6] heat treatment,[7] action of enzyme inhibitors,[8] and the presence of additives as saturated or insaturated alcohols,[3,9] Me_2SO,[9] tioacetamide, or adenine.[9] Only recently we reported a new method for the kinetic resolution of racemic 1-heteroaryl and 1-aryl ethanols *via* oxidation with bakers' yeast.[10] In comparison with the reduction of ketones that gives the prevalence of the *S*-enantiomer, the oxidation of the racemic alcohols with bakers' yeast in phosphate buffer affords the corresponding *R*-enantiomer in good enantiomeric excess.

On the basis of these considerations, in this paper we studied the BY-redox biotransformations varying both carbon source and chemical environments. Taking into consideration that the nature and the concentration of the carbon source affect the metabolic attitude of the yeast and can induce, as in the case of the glucose, repression of the synthesis of various enzymes,[11] different energy sources were supplemented to the yeast in order to study their influence on the yield and the enantioselectivity of the reduction of heteroaryl and aryl methyl ketones **1** and the oxidation of their racemic carbinols **2** (Scheme 1).

Scheme 1



a, Ar = 2-furyl; **b**, Ar = 2-thienyl; **c**, Ar = phenyl; **d**, Ar = 3-pyridyl

Table 1. Reduction with BY of the Ketones 1a–d

ketone	reaction condns	*t*/days	product (% yield)	% ee (abs confn)
1a	glucose	5	**2a** (7)	20 (*S*)
	phosphate buffer pH 5	5	**2a** (7)	80 (*S*)
	sodium citrate	7	**2a** (15)	60 (*R*)
	sodium lactate	7	**2a** (10)	50 (*R*)
1b	glucose	5	**2b** (14)	22 (*S*)
	sodium succinate	3	**2b** (10)	86 (*S*)
	sodium lactate	3	**2b** (10)	82 (*S*)
1c	glucose	4	**2c** (90)	100 (*S*)
1d	glucose	6	**2d** (100)	76 (*S*)

Table 2. Resolution of the Racemic Alcohols 2a–d *via* Oxidation with BY

alcohol	reaction condns	*t*/days	product (% yield)	% ee (abs confn)
2a	phosphate buffer pH 6	7	**2a** (39)	90 (*R*)
	CH_3COONH_4	7	**2a** (17)	100 (*S*)
	glucose	5	**2a** (51)	66 (*R*)
2b	CH_3COONa	5	**2b** (59)	38 (*S*)
	CH_3COONH_4	7	**2b** (21)	100 (*R*)
	sodium citrate	5	**2b** (60)	18 (*S*)
2c	CH_3COONa	6	**2c** (70)	18 (*S*)
	CH_3COONH_4	8	**2c** (41)	100 (*R*)
	sodium citrate	8	**2c** (47)	72 (*S*)
2d	sodium succinate	8	**2d** (39)	74 (*R*)

Both reduction and oxidation reactions are carried out by preparing a suspension of BY in tap water containing the selected carbon source[12] (2.5%) or suspending BY directly in phosphate buffer. All the reactions are monitored periodically by GLC on a chiral column (see Experimental Section). The significative results of the redox reactions are summarized in Tables 1 and 2.

In the presence of glucose (2.5%), the reductions of acetophenone (**1c**) and 3-acetylpyridine (**1d**) gave excellent yields (90–100%) with high enantiomeric excesses of the the *S*-enantiomers **2c** (≥95%) and **2d** (76%), respectively (Table 1). No inversion of configuration was obtained for these compounds varying the carbon source or the chemical enviroment. On the other hand, under the same conditions 2-acetylfuran (**1a**) and 2-acetylthiophene (**1b**) are scarcely reduced (7–14% yield) with low enantioselectivity (ee 20–22% of the *S*-enantiomer). In the case of 2-acetylfuran, the variation of the chemical enviroment (phosphate buffer

† Università di Ferrara.
‡ Università di Bologna.

(1) (a) Servi, S. *Synthesis* **1990**, 1. (b) Csuk, R.; Glanzer, B. I. *Chem. Rev.* **1991**, *91*, 49.
(2) Fuganti, C.; Grasselli, P.; Poli, G.; Servi, S.; Zorzella, A. *J. Chem. Soc., Chem. Commun.* **1988**, 1619.
(3) Nakamura, K.; Kawai, Y.; Oka, S.; Ohno, A. *Bull. Chem. Soc. Jpn.* **1989**, *62*, 875.
(4) Bucciarelli, M.; Forni, A.; Moretti, I.; Torre, G. *Synthesis* **1983**, 897.
(5) Wipf, R.; Kupfer, E.; Bertazzi, R.; Leuenberger, H. G. W. *Helv. Chim. Acta* **1983**, *66*, 485.
(6) Nakamura, K.; Higaki, M.; Ushio, M.; Oka, S.; Ohno, A. *Tetrahedron Lett.* **1985**, *26*, 4213.
(7) Nakamura, K.; Yasushi, K.; Ohno, A. *Tetrahedron Lett.* **1991**, *32*, 2927.
(8) Nakamura, K.; Kawai, Y.; Miyai, T.; Ohno, A. *Tetrahedron Lett.* **1990**, *31*, 3631.
(9) Naoshima, Y.; Maeda, J.; Munakata, Y. *J. Chem. Soc., Perkin Trans. 1* **1992**, 659.
(10) Fantin, G.; Fogagnolo, M.; Medici, P.; Pedrini, P.; Poli, S.; Sinigaglia, M. *Tetrahedron Lett.* **1993**, *34*, 883.
(11) Gancedo, C.; Serrano, R. *The Yeasts*; Rose, A. H., Harrison, J. S., Eds.; Academic Press: London, 1989; Vol. 3, Chapter 6.

(12) The carbon sources tested for all the substrates both in reduction and oxidation are as follows: glucose, sodium acetate, ammonium acetate, sodium citrate sodium succinate, and sodium lactate.

0022-3263/94/1959-0924$04.50/0

pH 5) increased the enantioselectivity of the reduction (ee 80% of the *S*-enantiomer) while the change of the carbon source (sodium citrate and lactate) produced the *R*-enantiomer with good enantioselectivity (60–50%, respectively). Moreover, in the reduction of 2-acetylthiophene (**2b**), the presence of sodium succinate and lactate drastically increased the enantiomeric excess of the *S*-enantiomer (86 and 82%, respectively).

If the stereocontrol of the reduction of the aryl methyl ketones **1a–d** is only partially affected by the variation of the carbon source and/or chemical enviroment, more interesting results are obtained in the kinetic resolution of the corresponding racemic alcohol **2a–d** by oxidation. As previously reported[10] the resolution of these compounds *via* oxidation with BY in phosphate buffer (pH range 5–7.2) afforded only the *R*-enantiomer with quite good to excellent ee (40–100%). It is worth mentioning that the oxidation, unlike the reduction, in the presence of glucose did not occur except for the alcohol **2a** which was resolved with ee 66% of the *R*-enantiomer. 2-Furylmethylcarbinol (**2a**) was also resolved in phosphate buffer at pH 6 (ee 90% of the *R*-enantiomer), while in the presence of ammonium acetate we had a complete inversion of configuration (ee 100% of the *S*-enantiomer). Excellent results, moreover, were obtained in the presence of ammonium acetate with the alcohols **2b** and **2c** (ee 100%), but the *R*-enantiomer was left. On the other hand, both **2b** and **2c** in the presence of sodium acetate and sodium citrate were oxidized to leave the *S*-enantiomer with discrete enantiomeric excess (18%–72%). Regarding the alcohol **2d** the *R*-enantiomer with good ee (74%) was obtained in the presence of sodium succinate.

On the basis of these data it is possible to stress some considerations on the BY-mediated redox biotransformations not only regarding the influence of the culture medium (carbon sources and chemical enviroments) but also of the structure of the compounds utilized for these reactions. In general, we can point out that BY reduces the ketones **1a** and **1b** (Ar is a five-membered ring) with low yields and poor or quite good enantiomeric excesses while under the same conditions the ketones **1c** and **1d** (Ar is a six-membered ring) are reduced with higher yields and normally good enantiomeric excesses. Practically in all cases the reductions afforded the *S*-enantiomers. The only exception is the reduction of 2-acetylfuran in the presence of sodium citrate that gave the *R*-enantiomer. On the contrary, the influence of the carbon sources and chemical enviroments on the yields and enantiomeric excesses of the oxidation reactions is considerable. We can observe that the alcohols with a five-membered aromatic ring (**2a** and **2b**, respectively) are oxidized more quickly than the alcohols with a six-membered aromatic ring **2c** and **2d**. Unlike the reduction, the oxidation is much affected by the variation of the chemical enviroments and carbon sources. In the presence of glucose, which is the favorite carbon source for the reduction, the oxidation of the alcohols **2b**, **2c**, and **2d** did not occur. The best results were obtained with the presence of ammonium acetate that resolved the alcohols **2a**, **2b**, and **2c**. With this carbon source and the presence of NH_4^+ ion we also obtained the inversion of the enantiomeric excess for the compound **2a** (*S*-enantiomer instead of the *R*-enantiomer). Similar behavior was observed in the presence of sodium acetate for the alcohols **2b** and **2c**, although with poor enantiomeric excesses.

Experimental Section

General. Gas chromatographic analyses were performed on a Carlo Erba HRGC 5160 Mega series chromatograph. 2-Acetylfuran, 2-acetylthiophene, 3-acetylpyridine, and acetophenone are commercially available (Aldrich). The corresponding racemic alcohols were prepared by reduction with sodium borohydride.

Enantiomer separation was obtained on a Megadex 1 column (25 m × 0.32 mm) containing permethylated β-cyclodextrine in OV 1701 from Mega snc: carrier gas helium (0.5 atm); for Ar = 2-thienyl and 3-pyridyl, temperature, 150–200 °C (2 °C/min); for Ar = 2-furyl, temperature, 110–200 °C (1.5 °C/min); for Ar = phenyl, temperature, 130–200 °C (2 °C/min). Retention time in min: **1a**, 5.64, (*R*)-**2a**, 8.03 and (*S*)-**2a**, 8.28; **1b**, 4.96, (*R*)-**2b**, 5.4 and (**S**)-**2b**, 5.55; **1c**, 5.66, (*R*)-**2c**, 8.35 and (*S*)-**2c** 8.71; **1d**, 4.42, (*R*)-**2d**, 9.08 and (*S*)-**2d** 9.30.

Redox Reaction with Bakers' Yeast. General Procedure. To a suspension of bakers' yeast (4 g) in a solution of tap water (20 mL) containing 0.5 g (2.5%) of glucose or of the selected organic salt or in the proper phosphate buffer solution (20 mL) (see Tables 1 and 2), the substrate (racemic alcohol **1** or ketone **2**) (12 mmol) in DMF (0.1 mL) was added. The suspension was stirred at 30 °C for the appropriate time. Aliquots (1 mL) were withdrawn periodically, extracted with diethyl ether (2 mL), and dried over anhydrous Na_2SO_4, and their GLC chromatograms on a chiral column were obtained.