Stereochemical Control of Bakers' Yeast Mediated Reduction of a Protected 2-Hydroxy Ketone

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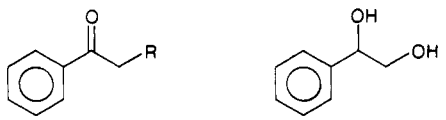
It is now widely accepted that bakers' yeast mediated bioreductions constitute a versatile method for the preparation of various chiral compounds of high enantiomeric excess (ee). The list of carbonyl compounds which can be reduced by the yeast is continuously growing and in most cases the stereochemical outcome of the bioreduction is well explained by the Prelog's rule.[1] However, a few exceptions have been found, and these are in some instances explained by assuming that the substrate is reduced by an alcohol dehydrogenase which does not proceed according to the above rule. For example, bakers' yeast reduction of 1-hydroxy acetone (**1a**) to (*R*)-(−)-1,2-propanediol (**2a**)[2] and of a few aliphatic 1-hydroxy ketones to (*R*)-1,2-diols[3] is apparently in contrast with the Prelog's rule. Recently, Whitesides has shown that simple 1-

1 **2**

a, R=H
b, R=$CH_2C_6H_5$

hydroxy ketones are good substrates for glycerol dehydrogenase of microbial source and that the *R* configuration of the enzymatically produced diols is identical with that obtained by bakers' yeast fermentation.[4] Bakers' yeast reduction of phenacyl alcohol (**3a**) affords (*R*)-1-phenyl-1,2-ethanediol (**4**),[5] and this configuration is in accord with Prelog's rule and could be derived from the catalysis of a glycerol dehydrogenase as well. In some

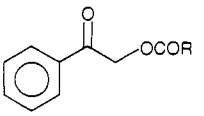


3a, R=OH
b, R=Br

4

microbial transformations, it is possible to obtain compounds of the desired stereochemistry by modifications of the substrate. Several examples of stereocontrolled reductions by bakers' yeast on suitably modified substrates have been reported.[6] For instance, we have shown[7] that the reduction of a protected 1-hydroxy ketone, namely, 1-(benzyloxy)-2-propanone (**1b**), afforded (*S*)-(+)-1,2-propanediol, 1-benzyl ether (**2b**). The stereochemical outcome of this biotransformation is opposite to the yeast-mediated reduction of 1-hydroxyacetone (**1a**).[2]

In order to investigate the stereochemistry of the bioreduction of a protected phenacyl alcohol (**3a**), we prepared a few esters of the hydroxy ketone **3a**. The choice of the protecting group could be dependent on the size of substituent R in formula **5**, since it has been already shown that several bulky ketones are not accepted by the conventional yeast dehydrogenase(s).[8]



5a, R=C_6H_5
b, R=H
c, R=CH_3
d, R=CH_2CH_3
e, R=$CH_2CH_2CH_3$

We started with the aim of preparing samples of the *R*-diol **4** by bakers' yeast reduction of phenacyl alcohol (**3a**) as reported by Ridley.[5] Modifications of the described incubation procedure[5] were needed because, in our hands, the compound **3a** was not a ready substrate for the bioreduction (20% of isolated product **4** after 1–2 days reaction). Finally, we were able to carry out the biotransformation enhancing the yields of the *R* diol **4** from the reported 45% to 85%, α_D −48.5° (*c* 2, acetone) (lit.[5] α_D −45.8°, same conditions). In order to evaluate the optical purity of the *R*-diol **4** prepared by the above method,[9] the optical rotation of our product was compared with a defined literature value. Mosher[10] recorded α_D −39.7° (*c* 4.33, ethanol) for a sample of 98 ± 2% optically pure *R*-(−)-diol **4** prepared from 98 ± 1% optically pure mandelic acid. *R*-(−)-Diol **4** prepared by us via bakers' yeast reduction exhibited α_D −37.3° in ethanol at the same concentration value, thus showing a 92% optical purity. Next, we prepared the benzoate **5a**, which, as expected, was not reduced by fermenting bakers' yeast, probably due to severe steric hindrance of the carbonyl substituents. We intended to use the smallest ester of hydroxy ketone **3a**, i.e., the formate **5b**, but its sensitivity to hydrolysis shown during its preparation led us to exclude this ester from further experiments. We then prepared the acetate **5c**, the propanoate **5d**, and the butanoate **5e**[11] either by replacement of bromine in the 2-bromo ketone **3b** with the proper carboxylate salts in polyethylene glycol (PEG) 400[12] or by esterification of phenacyl alcohol (**3a**). Incubation of the above esters with bakers' yeast led to the results summarized in the Table I. Protected diols esterified at the primary hydroxy group, 2-acyl derivatives **6**, were the main products. Thus, the 2-acetate **6a** and 2-propanoate **6b** were isolated and their ee, determined by the 200-MHz ^1H NMR of the corresponding MTPA esters,[13] were 94% and 91%, respectively. The *S* configuration was easily established by hydrolysis of the 2-esters to the corresponding *S*-(+)-diol **4**. The chromatographic properties (TLC,

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(8) MacLeod, R.; Prosser, H.; Fikentscher, L.; Lanyi, J.; Mosher, H. S. *Biochemistry* **1964**, *3*, 838.
(9) It should be mentioned that in ref 5 the reported value of optical rotation, −45.8° (*c* 2, acetone), is compared with a value of α_D −39.9° (*c* 6.6, acetone), but no literature reference is given. Other values of optical rotations for the diol **4** are reported by other authors (ref 3a and related citations; ref 10). However, only Dale and Mosher (ref 10) have quantitatively estimated the ee of diol **4** by ^{19}F NMR.
(10) Dale, J. A.; Mosher, H. S. *J. Org. Chem.* **1970**, *35*, 4002.
(11) The octanoate of compound **3a** was also prepared and incubated but was not a good substrate. The recovery of products was low (35%), and the main product was the starting ester (20%). Only 7% of 2-protected diol could be isolated together with 8% of diol **4** of low optical purity (α_D +5°).
(12) Santaniello, E.; Manzocchi, A.; Sozzani, P. *Tetrahedron Lett.* **1979**, 4581.
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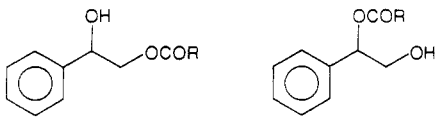

6 7

a, R=CH_3
b, R=CH_2CH_3
c, R=$(CH_2)_2CH_3$

column chromatography) of 2-acyl derivatives **6** were different from those of the 1-acyl derivatives **7**, so that we could isolate also the 1-protected diols **7a**, **7b**, and **7c**.[14] The above 1-protected diols may derive from an intramolecular transesterification, although it is not clear if such a process is chemically or enzymatically mediated. The table shows also that recovery of products becomes lower as longer is the chain in the ester moiety: this may be attributable to ester hydrolysis which becomes a more consistent reaction for the butanoate **5e**.[15] Moreover, in all incubations phenacyl alcohol (**3a**) and chiral diol **4** were always recovered. The modest optical purity of the diol **4** formed in the above biotransformations (21–58%) can be explained by the fact that this compound can actually be the mixture of products formed according to two bioreduction pathways. In fact, enzymatic hydrolysis of esters **5c–e** to phenacyl alcohol (**3a**) and following reduction, should lead to *R*-(−)-diol **4**, whereas hydrolysis of chiral 2- or 1-acyl derivatives **6** and **7** should furnish *S*-(+)-diol **4**. The fact that diol **4** obtained from the above incubations always present a positive optical rotation should indicate that the major biotransformation of the protected hydroxy ketones **5c–e** is the reduction of the carbonyl group occuring with a *S* stereochemistry.

In conclusion, we have shown that bakers' yeast reduction of esters of phenacyl alcohol (**3a**) leads to products with *S* configuration and that the stereochemistry of this biotransformation is opposite to the reduction of phenacyl alcohol itself, which affords the *R*-diol **4**. This result could be explained assuming that, following the Prelog's rule, in the compounds **5c–e** the ester moiety is "effectively larger"[16] than the phenyl group. Instead, in phenacyl alcohol (**3a**) the hydroxymethyl group should be "effectively smaller" than the aromatic group. On the other hand, at the present state of the knowledge on the presence of different oxidoreductases in bakers' yeast, it is not possible to exclude that phenacyl alcohol esters are substrates of an alcohol dehydrogenase different from that operating on phenacyl alcohol itself. In any event, our findings show that by bakers' yeast reduction of the suitable compound, it is now possible to prepare both *R*- and *S*-diols **4** in good yields (70–85%) and excellent optical purity (92–94% ee).

Experimental Section

Unless otherwise stated, materials are obtained from commercial suppliers and used without further purification. Bakers' yeast was from ERIDANIA (Italy). Unless otherwise stated, ^1H NMR spectra refer to 60-MHz spectra recorded on a Varian EM 360 L spectrometer for solutions in CCl_4, using $SiMe_4$ as internal standard. ^1H NMR (200-MHz) spectra were recorded in $CDCl_3$ on a Varian XL 200 spectrometer. Optical rotations were measured on a Perkin-Elmer Model 241 polarimeter. Distillation for analytical purposes were performed on a glass tube oven Buchi GKR-50. TLC analyses were carried out on silica gel Merck 60 F_{254} plates and column chromatographies were performed on silica gel Merck 60 (230–400 mesh).

Bakers' Yeast Reduction of 2-Hydroxyacetophenone (Phenacyl Alcohol) (3a). To a slurry of fermenting bakers' yeast in an open flask (88 g of yeast and 88 g of sucrose in 880 mL of tap water) was added 600 mg (4.4 mmol) of 2-hydroxyacetophenone (**3a**) in small portions, during a 4-h period with stirring at 30 °C. The mixture was stirred at 30 °C for 7 days and then cooled. After the addition of diethyl ether (100 mL) and Celite (50 g), stirring was continued for 15 min, and then the mixture was filtered through a sintered funnel. The solid was washed with ether (2 × 50 mL). The aqueous filtrate was extracted with diethyl ether (5 × 100 mL); the combined organic phases were dried over sodium sulfate, and evaporated at reduced pressure to give a solid residue (0.95 g), which was chromatographed on silica gel (20% acetone in dichloromethane).

(*R*)-(−)-Phenylethanediol **4** was obtained (0.52 g, 85% yield) with GLC, IR, and NMR identical with those of an authentic sample: α_D − 37.3° (*c* 4.33, EtOH) [lit.[9] α_D −39.7° (same concentration)].

2-Acetoxy-1-phenylethanone (5c). To a solution of dry potassium acetate (2.46 g, 25 mmol) in polyethylene glycol 400 dried on granular anhydrous calcium sulfate (14.5 g) was added phenacyl bromide (**3b**) (5 g, 25 mmol) and the mixture stirred for 30 min at 100 °C. After the mixture cooled, water (150 ml) was added, and the mixture was extracted with ether (4 × 80 mL). The organic extracts were washed twice with water, dried over sodium sulfate, and evaporated to yield 4 g of almost pure product **5c**, which was crystallized from diethyl ether–hexane to yield 3.6 g (80%): mp 49.5 °C (lit.[17] mp 49–50 °C); ^1H NMR δ 2.15 (3 H, s), 5.24 (2 H, s), 7.40–8.10 (5 H, Ar complex); IR ($CHCl_3$) 1748, 1702 cm^{-1}. Anal. Calcd for $C_{10}H_{10}O_3$: C, 67.4; H, 5.65. Found C, 67.5; H, 5.7.

Bakers' Yeast Reduction of 2-Acetoxy-1-phenylethanone (5c). To a slurry of fermenting bakers' yeast (7.52 g of yeast, 11.28 g of sucrose, 56.5 mL of tap water) was added 2-acetoxyacetophenone (**5c**) (0.67 g, 3.76 mmol). The mixture was stirred at room temperature for 8 h, then filtered through a Celite pad, and extracted with diethyl ether (4 × 30 mL). The extracts were dried over sodium sulfate and evaporated at reduced pressure, leaving a residue (0.655 g), which was purified by silica gel column chromatography (petroleum ether/ethyl acetate, from 85:15 to 1:1). The following products were obtained.

2-Acetoxy-1-phenylethanone (5c): yield, 0.02 g (3%); physical and chemical characteristics corresponding to those of an authentic sample.

2-Hydroxy-1-phenylethanone (3a): yield, 0.02 g (4%); physical and chemical characteristics corresponding to those of an authentic sample.

(*S*)-(+)-2-Acetoxy-1-phenylethanol (6a): yield, 0.47 g (70%); bp 130 °C (1.2 mmHg) [lit.[18] bp 135–136 °C (1.5 mmHg)]; ^1H NMR δ 1.97 (3 H, s), 3.35 (1 H, br s), 4.05–4.25 (2 H, m), 4.85 (1 H, q, *J* = 4 Hz), 7.30 (5 H, s);[19] IR (neat) 3450, 1725 cm^{-1}; α_D +28° (*c* 2, acetone). Anal. Calcd for $C_{10}H_{12}O_3$: C, 66.65; H, 6.7. Found: C, 67.8; H, 6.8.

A sample of the compound **6a** was transformed into the (*R*)-(+)-MTPA ester,[13] and 94% ee was established from integration of the signals of acetoxy and methoxy groups in its 200-MHz ^1H NMR spectrum. The methyl group of the acetoxy moiety showed two singlets centered at 1.98 and 2.05 ppm (ratio 1:1 and 97:3 for the derivatives from the racemic and optically active **6a**, respectively). The same ratios were observed for the two quartets

(14) TLC and column separation was achieved better for acetates than for propanoates. The most difficult separation was realized for the butanoates.

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(16) A similar example has been reported in the microbial reduction of α-haloaryl ketones by *Cryptococcus macerans*: Imuta, M.; Kawai, K.; Ziffer, H. *J. Org. Chem.* **1980**, *45*, 3352. Here, the α-halomethine in 2-bromoindan-1-one is "effectively larger" than the fused aromatic moiety, whereas the halomethyl group is "effectively smaller" than the aromatic ring in phenacyl bromide. We thank one of the referees for bringing this point to our attention.

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Table I. Bakers' Yeast Reduction of Esters of Phenacyl Alcohol (3a)

substrate	incubation time, h	recovery, %	products: 1-acyl[b] compd	1-acyl[b] yield,[a] %	diol 4[b] yield,[a] %	diol 4[b] ee,[c] %	2-acyl[b] compd	2-acyl[b] yield,[a] %	2-acyl[b] ee,[d] %
5c	8	97	**7a**	7	13	58	**6a**	70	94
5d	8	85	**7b**	8	4	48	**6b**	50	91
5d	20	82	**7b**	15	19	*e*	**6b**	40	*e*
5e	24	30	**7c**	2	5	21	**6c**	14	*e*

[a] Yields of products purified by silica gel column chromatography. [b] Configuration *S* for all products. [c] Optical purities determined by comparison with the literature values (ref 10). [d] Determined by 200-MHz ^{1}H-NMR analysis of (*R*)-(+)-MTPA esters. [e] Not determined for lack of pure material.

of the methoxy group centered at 3.47 and 3.58 ppm.

1-Phenyl-1,2-ethanediol (4) from (*S*)-(+)-6a. A sample of the (*S*)-(+)-2-acetate **6a** (0.09 g, 0.5 mmol) was reduced in anhydrous tetrahydrofuran (2 mL) with $LiAlH_4$ (0.06 g, 1.5 mmol) at −5 °C. After 0.5 h, 0.06 mL of water, 0.06 mL of 15% sodium hydroxide, 0.18 mL of water, were sequentially added, and the mixture was stirred for 10 min. The mixture was filtered and the precipitate washed with anhydrous diethyl ether. The filtrates were dried over sodium sulfate and evaporated. Benzene (2 mL) was added, and evaporation of the solvent gave dry *S*-diol **4** (0.075 g), pure by TLC and GLC: α_D +40° (*c* 2, acetone).

(+)-2-Acetoxy-2-phenylethanol (7a): yield, 0.048 g (8%); ^{1}H NMr δ 2.03 (3 H, s), 2.8 (1 H, br s), 3.8 (2 H, t, *J* = 6 Hz), 5.8 (1 H, t, *J* = 6 Hz), 7.3 (5 H, s); α_D +43° (*c* 2, acetone).

(*S*)-(+)-1-Phenyl-1,2-ethanediol (4): yield, 0.07 g (13%); α_D +28° (*c* 2, acetone); chromatographic and spectroscopic data identical with those of an authentic sample.

2-(Propanoyloxy)-1-phenylethanone (5d). A solution of phenacyl alcohol **3a** (1.5 g, 11 mmol) and propionic anhydride (1.5 mL) in dry pyridine (15 mL) was kept at room temperature (12 h). After being poured into water (50 mL), the ester **5d** was recovered by decanting the water layer and taken up with diethyl ether (80 mL). After washing with 0.5 N HCl (10 mL) and brine (10 mL), the organic extract was dried over sodium sulfate and evaporated. The residue was purified by filtration through a short silica gel column, by eluting with petroleum ether/ethyl acetate (95:5). Pure propanoate **5d** was obtained (1.7 g, 80%). A sample for analytical purposes was distilled: bp 210 °C (14 mmHg); IR (neat) 1750, 1705 cm^{-1}; ^{1}H NMR δ 1.17 (3 H, t, *J* = 8 Hz), 2.44 (2 H, q, *J* = 8 Hz), 5.24 (2 H, s), 7.40–8.20 (5 H, Ar). Anal. Calcd for $C_{11}H_{12}O_3$: C, 68.7; H, 6.3. Found: C, 68.85; H, 6.4.

Bakers' Yeast Incubation of 2-(Propanoyloxy)-1-phenylethanone (5d). Incubation conditions were as for the acetate **5c**, with incubation time of 8 h. Starting from 0.72 g of compound **5d**, after workup and silica gel chromatography (petroleum ether/ethyl acetate, from 85:15 to 1:1 as eluant), yields of purified products were as follows.

2-(Propanoyloxy)-1-phenylethanone (5d): yield, 0.122 g (17%) physical and chemical characteristics corresponding to those of an authentic sample.

2-Hydroxy-1-phenylethanone (3a): yield, 0.03 g (6%); physical and chemical characteristics corresponding to those of an authentic sample.

(*S*)-(+)-2-(Propanoyloxy)-1-phenylethanol (6b): yield, 0.365 g (50%); bp 180–185 °C (14 mmHg); IR (neat) 3450, 1725 cm^{-1}; ^{1}H NMR δ 1.10 (3 H, t, *J* = 7 Hz), 2.30 (2 H, q, *J* = 7 Hz), 3.30 (1 H, br s), 4.05–4.28 (2 H, m), 4.87 (1 H, m), 7.30 (5 H, s, Ar); α_D +23° (*c* 2, acetone). Anal. Calcd for $C_{11}H_{14}O_3$: C, 68.0; H, 7.25. Found: C, 68.15; H, 7.4.

A sample of the compound **6b** was transformed into the (*R*)-(+)-MTPA ester[13] and 91% ee was established from integrations of the signals of methoxy group in its 200-MHz ^{1}H NMR spectrum. Two multiplets at δ 3.47 and 3.58 were present for the derivative from the racemic and optically active **4d** at ratio 1:1 and 95.5:4.5, respectively.

(+)-2-(Propanoyloxy)-2-phenylethanol (7b): yield, 0.06 g (8%); ^{1}H NMR δ 1.12 (3 H, t, *J* = 7 Hz), 2.40 (2 H, q, *J* = 7 Hz), 2.60 (1 H, br s), 3.75 (2 H, d, *J* = 6 Hz), 5.85 (1 H, t, *J* = 6 Hz), 7.34 (5 H, s, Ar); α_D +58° (*c* 2, acetone).

(*S*)-(+)-1-Phenyl-1,2-ethanediol (4): yield, 0.02 g (4%); identical with an authentic sample; α_D +23° (*c* 2, acetone).

Acknowledgment. We thank Ministero della Pubblica Istruzione for financial help.

Registry No. **3a**, 582-24-1; **3b**, 70-11-1; **4**, 16355-00-3; **5c**, 2243-35-8; **5d**, 54797-42-1; **5e**, 54797-43-2; **6a**, 103574-67-0; **6b**, 115482-82-1; **6c**, 115482-83-2; **7a**, 115482-84-3; **7b**, 115482-85-4; **7c**, 115482-86-5.

Unusual Structure in Meisenheimer Complex Formation from the Highly Electrophilic 4,6-Dinitrobenzofuroxan

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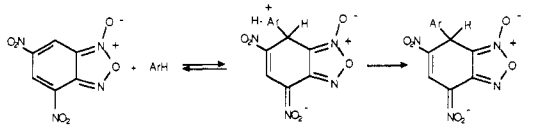
4,6-Dinitrobenzofuroxan (DNBF) exhibits an extremely high electrophilic character which is very useful to assess the reactivity of very weak nucleophiles.[1] Thus, many aromatic amines which have a very low carbon basicity undergo facile addition to DNBF to yield carbon-bonded σ-adducts as the thermodynamically stable species.[2,3] 1,8-Bis(dimethylamino)naphthalene, i.e., the Proton Sponge, is the most spectacular example in the series.[4] Also, weakly basic aromatics (ArH) like 1,3,5-trimethoxybenzene or indoles react with DNBF, affording the stable σ-adducts **1** according to eq 1.[5a] In these latter instances, the reactions proceed so readily that a detailed kinetic analysis of the formation of **1** could be made.[5b]



1

With the aim at assessing the reactivity of indene, we have looked at the interaction of this derivative with

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