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Stereoselectivity of Baker's Yeast Reduction of 2-Propanones: Influence of Substituents

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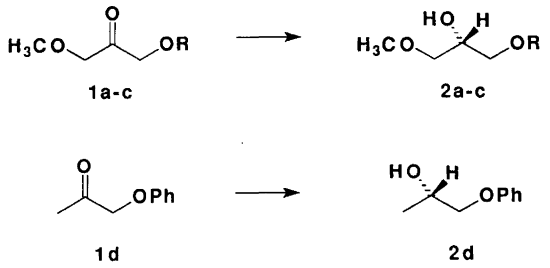
The stereoselectivity of Baker's yeast reduction of prochiral α-oxygenated 2-propanones has been studied by varying the substrate structure. The 1-hydroxy-3-methoxy-3-propanone **1a** was reduced to the corresponding alcohol (*R*)-**2a** with 88% enantiomeric excess. Replacing the hydroxy group in **1a** with phenoxy or benzyloxy (**1b** and **1c**) gave the alcohols (*S*)-**2b** and (*S*)-**2c** with 53 and 32% ee, respectively. Reduction of the methyl ketone **1d** gave the alcohol (*S*)-**2d** with 91% ee. Attempts to improve the enantioselectivity of the reduction of **1c** by lowering the substrate concentration or addition of selective reductase inhibitors had only small effect on the enantioselectivity.

Biocatalytic methods, as alternative to traditional methods, have gained wide interest among organic chemists for the production of homochiral compounds.[1–7] The most frequently used methods are lipase-catalysed racemate resolutions, and Baker's yeast-catalysed enantioselective reductions of prochiral ketones.

The method of yeast reduction of prochiral ketones as a tool in synthetic organic chemistry goes back to the beginning of 1920s.[8] The pioneering work was performed on α-hydroxyacetone, which gave (*R*)-1,2-propanediol with more than 90% ee. Later work has shown that reduction of α-hydroxy ketones generally takes place with high enantioselectivity. For instance, structurally related acyloins of α-hydroxyacetone have been reduced exclusively to the corresponding diols with anti-Prelog[9] configuration.[10–13] Recent reviews clearly show that Baker's yeast has a large potential as a catalyst in organic chemistry, owing to ease of handling and broad substrate acceptability.[14–16]

We have previously reported the enzyme-catalysed production of homochiral derivatives of 3-chloro-1,2-propanediol[17] and 3-methoxy-1,2-propanediol.[18] The enantiomeric ratios (*E*)[2] obtained ranged from 15 to >100. While racemate resolutions are limited to 50% theoretical yield of a homochiral product, Baker's yeast (*Saccharomyces cerevisiae*) reduction of a prochiral ketone can be run to 100% conversion and still maintain a high enantiomeric excess in the product. The separation of unchanged substrate and product is avoided since the reaction can be run to complete conversion. We have therefore included this method in our attempts to produce homochiral C_3-compounds.

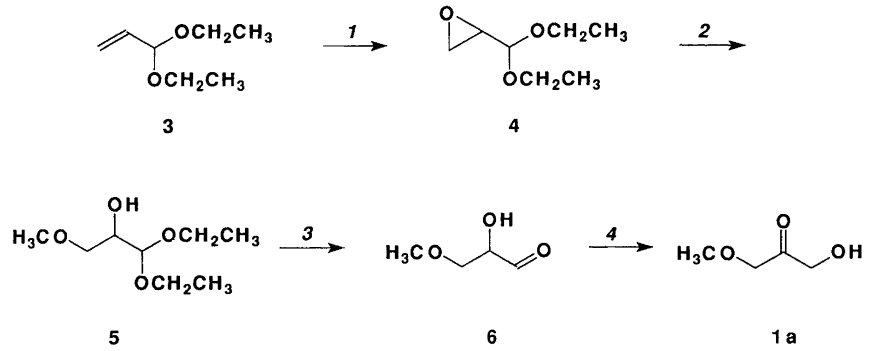


Scheme 1. Stereochemical course of Baker's yeast reduction of **1a–d**: **a**, R=H; **b**, R=Ph; **c**, R=CH_2Ph.

We here report Baker's yeast reduction of the α-hydroxy ketone **1a** to the corresponding diol, 3-methoxy-1,2-propanediol and the effects on the yeast enantioselectivity by replacing the hydroxy group with phenoxy or benzyloxy, i.e., **1b** and **1c** (Scheme 1). Since size discrimination is believed to be of major importance in yeast enantioselection,[9,19] we have also included the methyl ketone **1d** to introduce lager differences in size between the substituents.

Results and discussion

The racemic alcohols **2b** and **2c** were synthesized as previously described from epichlorohydrin and the appropriate alcohol.[18] Oxidation of **2b** to ketone **1b** was accomplished by Jones oxidation. However, attempts to apply chromium oxidation of **2c** (Jones or PCC) gave considerable amounts of by-products, probably due to



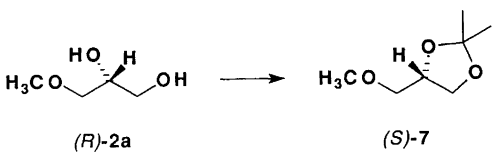
Scheme 2. Synthesis of the hydroxy ketone **1a**: *1*, 30% H_2O_2, $KHCO_3$, MeOH–PhCN; *2*, MeOH–NaOH; *3*, Dowex 50 (H^+); *4*, triethylamine, dioxane.

oxidation/cleavage of the benzylic ether. Swern oxidation, on the other hand, gave **1c** in satisfactory yield (58%).

The ketone **1a** was synthesized in four steps from acrolein diethylacetal (**3**) (Scheme 2). Epoxidation of **3** gave glycidaldehyde (oxiranecarbaldehyde) diethylacetal (**4**), which after treatment with sodium hydroxide in anhydrous methanol gave 3-*O*-methylglyceraldehyde diethyl acetal (**5**).[20] Compound **5** was then stirred with Dowex 50 (H^+) resin to give 3-*O*-methylglyceraldehyde (**6**) which exists as a dimer.[20] Isomerisation of **6** with triethylamine in dioxane gave the ketone **1a**.[20] Isomerisation of the aldehyde **6** to the ketone **1a** was attempted with immobilised glucose isomerase (Sweetzyme, Novo Nordisk). Glucose isomerase was added to the aldehyde **6** and dissolved in a solution of disodium maleate. The pH of the solution was maintained at 7.5 and approximately 55°C. However, after 48 h no trace of the isomerisation product **1a** was observed.

Reduction of the ketones **1a–d** was performed with an actively fermenting mixture, consisting of Baker's yeast and sucrose in phosphate buffer. The reactions were stopped by extraction with diethyl ether after 48 h. The crude products from reduction of **1b–d** were subjected to column chromatography which afforded (*S*)-**2b–d** in 53, 32 and 91% ee, respectively. The crude alcohol (*R*)-**2a**, from reduction of **1a**, was transformed into the corresponding acetonide (*S*)-**7** without further purification (Scheme 3). Subsequent chiral GLC analysis of the acetonide revealed that the diol (*R*)-**2a** was formed in 88% ee.

The absolute configurations of the alcohols produced were verified by comparison with reference compounds which were synthesized from homochiral C_3-building blocks. Homochiral (*S*)-**7** was synthesized by methylation of (*S*)-isopropylideneglycerol under basic conditions in dimethyl sulfoxide (DMSO) with methyl iodide. The synthesis of homochiral (*R*)-**2b** and (*R*)-**2c** were performed as previously reported[18] starting from (*S*)-glycidol (oxiranylmethanol) and (*S*)-epichlorohydrin, respectively. Homochiral (*S*)-**2d** was synthesized from (*S*)-propylene oxide by base-catalysed regioselective ring opening of the epoxide with phenol.



Scheme 3.

Previous investigations have shown that α-hydroxyacetone **8** is reduced to the corresponding (*R*)-diol with 90% ee[8] (Table 1). Reduction of the phenyl ether **1d** gave the corresponding alcohol (*S*)-**2d** with 91% ee. This change in absolute configuration accords with previous investigations which have shown that the benzyl ether **9** is reduced to the corresponding alcohol in 90% ee and with the *S*-configuration.[22] The absolute configuration of **2d** was initially estimated by comparison with the CD spectrum of the structurally related compound (*R*)-**9** thus suggesting that reduction of **1d** gave the corresponding alcohol with the *R*-configuration. However, analysis on a chiral HPLC column (Chiralcel-OB) revealed, from comparison with the synthesised reference compound (*S*)-**2d**, that the absolute configuration was *S* and not *R*.

The ketones **1a–c** were reduced with large differences in the yeast enantioselection, and the enantiomeric excesses obtained varied from 88 to 33%. The α-hydroxy ketone **1a** was reduced to the corresponding diol (*R*)-**2a** with anti-Prelog configuration (88% ee). Hence, the yeast enantioselection was not affected on going from **8** to **1a**. However, the methoxy-substituted compounds **1b** and **1c**

Table 1. Enantiomeric excesses and configurations obtained by yeast-catalysed reductions of ketones **1a–d**, **8**[8] and **9**.[21]

	R	%ee	Conf.		R	%ee	Conf.
8	H	90	*R*	**1a**	H	88	*R*
1d	Ph	91	*S*	**1b**	Ph	55	*S*
9	Bn	90	*S*	**1c**	Bn	33	*S*

were reduced with much lower enantioselection than the unsubstituted analogs, i.e., **1d** and **9** (Table 1).

Low enantioselection in the Baker's yeast reduction of a prochiral ketone may be due to the fact that the yeast contains competing reductases of opposite chirality.[23] It is, however, possible to introduce changes in the reaction conditions which may enhance low enantioselection. For instance, lowering the substrate concentration[23] or addition of selective inhibitors[24–26] against the *R*- or *S*-enzyme activities has been reported.

The synthetically useful benzyl ether from reduction of **1c** was obtained in only 33% ee under standard conditions. We attempted to optimise the yeast enantioselection by reducing the substrate concentration from 40 mM to 10 mM and by addition of the selective reductase inhibitors allyl alcohol and methyl vinyl ketone. However, the enantioselection was only slightly improved. Similar results were also obtained for the reduction of **1b**, i.e., the ee increased from 53 to 61% by lowering the substrate concentration from 40 to 5 mM. The lack of significant influence on the yeast enantioselection by these methods may indicate that the low selectivity for **1b** and **1c** stems from constricting interactions between the substrate and one reductase rather than the action of several enzymes of different chirality.[27]

Experimental

Chemicals. 1,2-*O*-Isopropylidene-*sn*-glycerol was purchased from Sigma, (*R*)-1,2-propanediol and (*S*)-propylene oxide from Fluka and 1-phenoxy-2-propanone from Aldrich. Solvents and other chemicals were of *purum* or *puriss.* quality unless otherwise stated.

Analytical methods. The enantiomeric excess (ee) of **7** was determined by chiral GLC analysis on a Chiraldex B-PH cyclodextrin column 2 psi with H_2 as the carrier gas. Temp. prog. 50°C ($t = 0$ min)–3° min^{-1}–150° ($t = 3$ min), $t_R(R)$ 5.71 min and $t_R(S)$ 6.07 min. The enantiomeric excess of **1b** was determined by a chiral GLC analysis after derivatisation with (*S*)-phenylethyl isocyanate, obtained from Fluka. GLC analysis was performed on DB-1701, 10 psi H_2-carrier gas. Temp. prog. **1b**, isothermal 260°C, $t_R(SR)$ 8.8 min and $t_R(RR)$ 9.0 min. The enantiomeric excess of **1c** and **1d** were determined by HPLC using a Varian 9000 system equipped with UV–VIS detector (2550) and a chiral column, Chiralcel OB, delivered by J. T. Baker, Deventer, Holland. Solvents: **1c**, hexane–EtOH = 90:10, 0.5 ml min^{-1}; **1d**, hexane–EtOH = 97:3, 0.25 ml min^{-1}. The ee-values were also determined by optical rotation using an Optical Activity Ltd. AA-10 automatic polarimeter, concentrations (*c*) are given in g/100 ml. CD spectra were recorded on an ISA Jobin Yvon *Division D'Instruments* Autodichrograph Mark IV-instrument connected to an IBM PC. ^1H and ^{13}C NMR spectra were recorded for $CDCl_3$ solutions using Me_4Si as an internal reference, shift values are in ppm. The instrument was a JEOL EX-400 operating at 400 MHz for ^1H and 100.4 MHz for ^{13}C. Mass spectra were measured using an AEI MS-902 instrument.

1-Hydroxy-3-methoxy-2-propanone **1a** was obtained in a four-step synthesis. Epoxidation of acrolein diethylacetal (**3**) with 30% aq. H_2O_2 in the presence of $KHCO_3$, MeOH and benzonitrile gave the diethylacetal **4**. ^1H NMR: δ 1.20 (3 H, t, OCH_2CH_3), 1.25 (3 H, t, OCH_2CH_3), 2.80 (2 H, d, oxirane), 3.10 (1 H, q, oxirane), 3.40–3.90 (4 H, m, $2 \times OCH_2CH_2CH_3$), 4.35 [1 H, d, $J = 4$ Hz, $CH(OEt)_2$]. Compound **4** furnished **5** after treatment with NaOH in anhyd. MeOH.[20] ^1H NMR: δ 1.20 (3 H, t, OCH_2CH_3), 1.25 (3 H, t, OCH_2CH_3), 2.55 (1 H, br, OH), 3.40 (3 H, s, OCH_3), 3.45–3.90 [7 H, m, $2 \times OCH_2CH_3$, $CH(OH)CH_2$], 4.50 (1 H, d, $J = 4$ Hz). Compound **5** was stirred with Dowex 50 (H^+) resin to give **6** as a dimer.[19] Isomerisation of **6** with Et_3N in dioxane gave **1a**.[21] ^1H NMR: δ 3.45 (3 H, s, OCH_3), 4.15 (2 H, s, H_3COCH_2), 4.42 [2 H, s, $C(=O)CH_2OH$]. Overall yield from **3**, 20%.

1-Phenoxy-3-methoxy-2-propanone **1b** *from* (rac)-**2b**. A solution of **2b** (4.016 g, 0.022 mol) in acetone (50 ml) was titrated with 8 M Jones reagent at room temperature until no more **2b** was visible on TLC. The solvent was removed at reduced pressure, and Et_2O (100 ml) was added. The Et_2O was filtered through a short pad of Florisil, extracted with H_2O (3×50 ml) and dried over $MgSO_4$. Distillation, $b.p._{0.5}$ 170–175°C, gave **1b** as a solid compound which was crystallised from hexane, m.p. 48–52°C, 36%. ^1H NMR: δ 4.33 [2 H, s, $C(=O)CH_2OCH_3$], 4.74 (2 H, s, $PhOCH_2$) and 3.46 (3 H, s, OCH_3). ^{13}C NMR 59.6 (q), 71.6 (t), 76.2 (t), 114.5 (2 d), 121.9 (d), 129.7 (2 d), 157.6 (s) and 204.2 (s). M^+ 180.0784, calc. for $C_{10}H_{12}O_3 \cdot 180.0786$.

1-Benzyloxy-3-methoxy-2-propanone **1c** *from* **2c**. A solution of freshly distilled oxalyl chloride (0.712 g 5.61 mmol) in CH_2Cl_2 (25 ml) was cooled to −70°C, and DMSO (0.957 g, 12.24 mmol) in CH_2Cl_2 (5 ml) was added over 5 min. The reaction mixture was stirred for further 10 min at the same temperature, and **2c** (1.0 g, 5.1 mmol) in CH_2Cl_2 (7 ml) was added over 5 min. The solution became cloudy upon addition of the alcohol. After additional stirring for 15 min, Et_3N (1.84 ml 25.5 mmol) was added and the cooling bath was removed. At room temperature H_2O (20 ml) was added and the organic phase was separated from the water phase which was extracted with CH_2Cl_2 (3×50 ml). The combined organic phases was extracted with 2% eq. HCl and sat. aq. $NaHCO_3$ and dried over $MgSO_4$ to give a yellow oil (0.8 g) which was chromatographed (silica gel; acetone–hexane = 1:3), yield 58%. ^1H NMR: δ 4.29 [2 H, s, $C(=O)CH_2OCH_3$), 4.28 (2 H, s, $BnOCH_2$), 4.68 (2 H, s, $PhCH_2$) and 3.50 (3 H, s, OCH_3). ^{13}C NMR: δ 59.4 (q), 73.5 (t), 73.6 (t), 76.2 (t), 127.9 (2 d), 128.1 (d),

128.5 (2 d), 137.0 (s) and 205.6 (s). IR (NaCl) 1735 cm^{-1}.

3-O-Methyl-1,2-O-isopropylidene-sn-glycerol (*S*)-**7**. To DMSO (15 ml) was added powdered KOH (30.0 mmol, 4 equiv.). The mixture was stirred for 15 min after which 1,2-*O*-isopropylidene-*sn*-glycerol (7.5 mmol, 1 equiv.) and MeI (15.0 mmol, 2 equiv.) were added. The suspension was stirred for 1 h and the mixture was poured into water (100 ml) and extracted with CH_2Cl_2 (5 × 100 ml). The combined organic phases were extracted with water (5 × 50 ml), dried and chromatographed (silica gel; acetone–hexane = 1:3), yield 41%. ^1H NMR: δ ABMXY-system for $CH_2CH(O)CH_2OCH_3$, 4.06 (1 H), 3.71 (1 H), 4.28 (1 H), 3.48 (1 H) and 3.41 (1 H), $J_{AB} = 8.0$, $J_{AM} = 6.4$, $J_{BM} = 6.4$, $J_{XM} = 6.0$, $J_{YM} = 5.2$ and $J_{XY} = 10.0$, 3.40 (3 H, s, OCH_3), 1.37 (3 H, s, CH_3) and 1.43 (3 H, s, CH_3). ^{13}C NMR: δ 25.4 (q), 26.8 (q), 59.4 (q), 66.7 (t), 73.8 (t), 74.6 (d) and 109.5 (s). $[\alpha]_D^{20} = +2.7$ (*c* 1.30 hexane) >99% ee.

(S)-*1-Phenoxy-2-propanol*, (S)-**2d**. To a stirred solution of KOH (0.3 g, 5.3 mmol) in 10 ml DMSO was added phenol (1.3 g, 14 mmol) in 10 ml DMSO. After 1 h (*S*)-propylene oxide (1 g, 17 mmol) was added and the reaction was stirred overnight by heating at 55°C. Extraction with Et_2O and distillation $b.p._{0.1} = 150°C$ afforded (*S*)-**2d**, yield 60%. ^1H NMR: δ $ABMX_3$-system for $OCH_2CH(O)CH_3$; 3.86 (1 H), 3.73 (1 H), 4.12 (1 H) and 1.21 (3 H), $J_{AB} = 9.2$, $J_{AM} = 3.0$, $J_{BM} = 7.6$, $J_{MX} = 6.8$, 2.45 (1 H, br, OH). ^{13}C NMR: δ 18.7 (q), 66.2 (d), 73.1 (t), 114.5 (2d), 121.0 (d), 129.5 (2d) and 158.5 (s). $[\alpha]_D^{20} = -2.7$ (*c* 1.80, EtOH) >99% ee.

(R)-*1-Benzyloxy-2-propanone*, (R)-**9**. To (*R*)-1,2-propanediol (0.88 g, 12 mmol) and KOH (1.1 g, 44 mmol) was added DMSO (5 ml). The mixture was stirred for 1 h after which benzyl chloride (1.5 g, 12 mmol) was added, followed by heating at 75°C for 2 h. Addition of H_2O and extraction with toluene gave a crude oil which was chromatographed (silica gel; CH_2Cl_2–Et_2O = 1:2), yield 20%. ^1H NMR: δ 1.10 (3 H, d, $J = 6$ Hz, $CHCH_3$), 2.55 (1 H, br, OH), 3.10–3.60 [2 H, m, $CH_2CH(OH)$], 3.75–4.15 [1 H, m, $CH_2CH(OH)CH_3$], 4.55 (2 H, s, $PhCH_2$). $[\alpha]_D^{20} = -17.6$ (*c* 14.3, CH_2Cl_2) >99% ee.

Reduction of prochiral ketones: general procedure. To a well stirred solution of sucrose (16 g) in 40 ml phosphate buffer (0.1 M) at 30°C were added 5 g dry Baker's yeast. The mixture was stirred vigorously for 30 min and the ketone (1.6 mmol) was added. The reaction mixture was shaken for 48 h at 30°C and then extracted with ether. The combined extracts were dried over anhydrous $MgSO_4$ and then evaporated. The crude oil was purified by column chromatography, unless otherwise indicated. ^1H and ^{13}C NMR spectra were identical with racemic samples.

(R)-*3-Methoxy-1,2-propanediol*, (R)-**2a**. The prochiral ketone **1a** was reduced with Baker's yeast to give the alcohol (*R*)-**2a**. The crude diol was transformed directly into the corresponding acetonide (*S*)-**7** by general-acid catalysis and analysed on a chiral GLC column, 88% ee.

(S)-*3-Methoxy-1-phenyl-1,2-propanediol*, (S)-**2b**. The prochiral ketone **1b** was reduced with Baker's yeast to give the alcohol **2b**. Column chromatography (acetone–hexane = 1:3), yield 35%. $[\alpha]_D^{20} = -1.3$ (*c* 1.11, EtOH), reference (*R*)-**2b** $[\alpha]_D^{20} = +4.2$ (*c* 1.67, MeOH). Analysed as diastereomeric carbamate derivatives, 53% ee.

(S)-*3-Methoxy-1-phenylmethyl-1,2-propanediol*, (S)-**2c**. The prochiral ketone **1c** was reduced with Baker's yeast to give alcohol **2c**. Column chromatography (acetone–toluene–hexane = 2:1:4), yield 32%. $[\alpha]_D^{20} = -1.3$ (*c* 1.54, EtOH), reference (*R*)-**1c** $[\alpha]_D^{20} = +5.6$ (*c* 1.07, MeOH), 32% ee (Chiralcel-OB).

(S)-*1-Phenoxy-2-propanol* (S)-**2d**. According to the general procedure, prochiral ketone **1d** was reduced with Baker's yeast to give alcohol **2d**. Column chromatography (Et_2O–CH_2Cl_2 = 1:3), yield 45%, 91% ee (Chiralcel-OB).

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