Control of the Enantioselectivity of the Bioreduction with Immobilized Bakers' Yeast in a Hexane Solvent System

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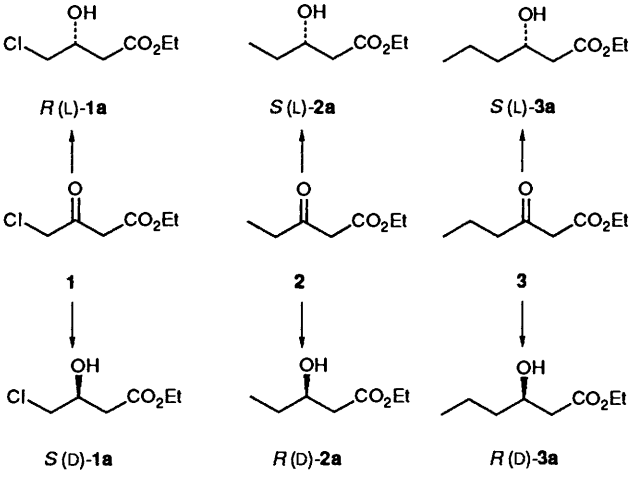
Enantioselective reduction of β-keto esters with immobilized bakers' yeast in hexane was either controlled or changed by additives such as saturated and unsaturated alcohols, Me_2SO, thioacetamide, or adenine, instead of glucose.

In recent years, bakers' yeast has been used for enantioselective reductions of β-keto esters, keto acids, or α,β-unsaturated carbonyl compounds.[1] Several ways for controlling the stereochemistry of aqueous bakers' yeast reductions have been adopted such as modification of substrate structure,[2] immobilization of the bakers' yeast,[3] or with additives such as allyl alcohol and metal salts.[4] More recently we reported that immobilized bakers' yeast (IBY) entrapped in calcium alginate beads functioned both in hexane and water systems, IBY-mediated reductions in the former, using alcohols as an energy source or additive, giving chemical and optical yields similar to those with analogous reductions using glucose.[5] In continuance of our work on IBY reactions, we found that enantioselective reduction of certain β-keto esters, **1**–**3**, in hexane, could be controlled by additives such as alcohols, Me_2SO, thioacetamide, or adenine, instead of glucose.

For 4-chloro-3-oxobutanoate **1**, each reduction in hexane, using thioacetamide, Me_2SO, and saturated alcohols (*e.g.* methanol, butan-2-ol, and 2,2,2-trifluoroethanol), gave the corresponding chiral hydroxy esters *R*(L)-**1a** with an enantiomeric purity of 21–43%. In contrast, for analogous reductions of **1** with allyl alcohol and quinine the stereochemistry of **1a** was largely shifted to the D-isomer, *S*(D)-**1a** of 55% ee being produced in the presence of the former additive. Both reductions of the keto ester **2**, using thioacetamide and adenine, yielded *S*(L)-**2a** with 55% ee, while that of **2** with allyl alcohol gave *R*(D)-**2a** with 64% ee. Similar reductions with saturated alcohols were also carried out, and resulted in *S*(L)-**2a** with an enantiomeric purity of 18–46%. For substrate **3**, the stereochemistry of the product (*R*)-**3a** obtained for reductions in the presence of several additives including saturated alcohols shifted towards the L-isomer, relative to that produced for the reduction using glucose; in contrast, the stereochemistry in Me_2SO and allyl alcohol systems was shifted more to the D-isomer than that in glucose system (see Table 1).



Scheme 1 Reactions in the presence of IBY, hexane and additives

A combination of DMSO and glucose was a particularly

Table 1 Enantioselectivity of the reduction of keto esters **1**–**3** with immobilized bakers' yeast.[a]

	1a			**2a**			**3a**		
Additive	% Yield	% Ee[b]	Config.[d]	% Yield	% Ee[c]	Config.[d]	% Yield	% Ee[c]	Config.[d]
Methanol	54	25	*R*(L)	45	18	*S*(L)	13	38	*R*(D)
Ethanol	64	21	*R*(L)	35	41	*S*(L)	10	62	*R*(D)
Propan-2-ol	65	28	*R*(L)	37	39	*S*(L)	11	47	*R*(D)
Butan-1-ol	70	30	*R*(L)	10	33	*S*(L)	5	46	*R*(D)
Butan-2-ol	70	35	*R*(L)	42	42	*S*(L)	1	42	*R*(D)
Trifluoroethanol	70	37	*R*(L)	40	46	*S*(L)	3	39	*R*(D)
Ethyl chloroacetate	56	26	*R*(L)	4	51	*S*(L)	3	—[f]	—
Me_2SO	37	32	*R*(L)	26	10	*S*(L)	8	78	*R*(D)
Thioacetamide	52	43	*R*(L)	21	55	*S*(L)			
Adenine	66	23	*R*(L)	22	55	*S*(L)	16	51	*R*(D)
Me_2SO + glucose[e]	20	8	*R*(L)	20	4	*R*(D)	31	99	*R*(D)
Quinine	51	36	*S*(D)	39	—[f]	—	36	63	*R*(D)
Allyl alcohol	51	55	*S*(D)	6	64	*R*(D)	6	95	*R*(D)
Glucose	34	4	*S*(D)	45	23	*S*(L)	35	67	*R*(D)
None	30	35	*R*(L)	30	22	*S*(L)	10	64	*R*(D)

[a] Reactions were run in the presence of an additive (1 g). [b] Determined by HPLC analysis of the MTPA ester derived from **1a** [GL Science Lichrosorb SI-100, diethyl ether–hexane (1:20), 1.0 cm^3 min^{-1}, 254 nm]. [c] Determined by HPLC analysis of the benzoate esters derived from **2a** and **3a** [Daicel Chiralcel OB, propan-2-ol–hexane (1:9), 0.7 cm^3 min^{-1}, 220 nm]. [d] Both the stereochemical designations of *RS* and DL were used to show clearly the direction of stereochemistry. [e] Me_2SO (10 g) and glucose (1 g) were used. [f] Racemic.

satisfactory additive, the enantiomeric purity of the product *R*(D)-**3a** reaching >99%. It is interesting to note that the degree of the change of these enantioselectivities varies, depending on the keto ester involved, whereas the direction of the selectivity is controllable by selecting the appropriate additive. In reductions using thioacetamide, adenine, and saturated alcohols including butan-2-ol and trifluoroethanol, for example, the stereochemistry of **1a**–**3a** was generally shifted to the L-isomer. All of these additives can be classified as being L-selective (or L-directing). Since with allyl alcohol, the stereochemistry was exclusively shifted to the D-isomer, this additive can be classified as being D-selective (or D-directing).

Although the present stereochemical control system on IBY-mediated reductions in hexane has not been optimized these results indicate that the enantioselective reductions with bakers' yeast may be controlled in both aqueous and organic solvent systems (particularly in hexane), by using appropriate L-selective or D-selective additives.

Experimental

Immobilized bakers' yeast entrapped in calcium alginate beads, *ca.* 1.5 mm diam., was prepared according to the procedure described previously.[5b] $[\alpha]_D$ Values were recorded in 10^{-1} deg cm^2 g^{-1}.

IBY Reduction of **1**–**3** *in the Presence of Adenine.*—IBY prepared from free bakers' yeast (10 g) was added to a solution containing the appropriate keto ester, **1**–**3** (1 g), adenine (1 g) and hexane (200 cm^3). Each mixture was shaken at 30–35 °C for a different period: 4 h for **1**, 52 h for **2** and 50 h for **3**. The reaction mixture was then filtered, and IBY beads were well washed with water; the filtrate and washings were combined and extracted with diethyl ether. Work-up of the extract gave a crude product, which was purified by column chromatography (silica gel and hexane–ethyl acetate) and microvacuum distillation to yield a chiral hydroxy ester; 0.668 g of **1a**, $[\alpha]_D^{22}$ +5.22 (*c* 6.12, $CHCl_3$), 0.223 g of **2a**, $[\alpha]_D^{22}$ +20.64 (*c* 3.85, $CHCl_3$) and 0.166 g of **3a**, $[\alpha]_D^{22}$ −11.91 (*c* 4.24, $CHCl_3$), were obtained, respectively. Each hydroxy ester was fully characterized by IR and ^1H NMR spectroscopy.

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