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Stereoselectivity of Yeast Reductions – an Improved Procedure for the Preparation of Ethyl (*S*)-3-Hydroxybutanoate and (*S*)-2-Hydroxymethylbutanoate

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Abstract: Ethyl 3-oxo- and 2-formyl-butanoate (4–5 g/L) are reduced in ca. 70% yield to the title compounds of > 90% enantiomeric excess by baker's yeast (125 g/L) which had been kept in 5% aqueous ethanol with shaking under aerobic conditions in the absence of sugar for four days.

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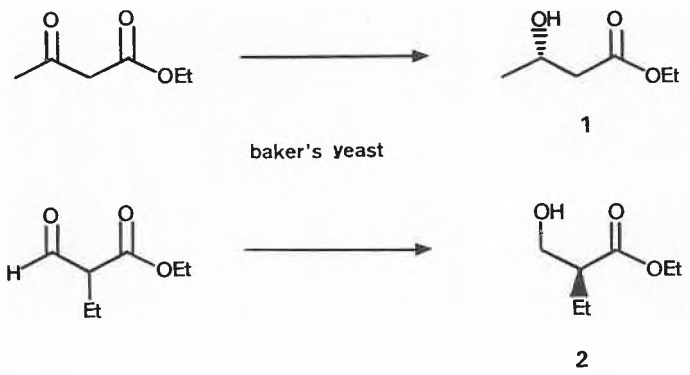
Enantioselective reduction by baker's yeast[1] (*Saccharomyces cerevisiae*) of acetoacetic acid derivatives produces (*S*)-3-hydroxybutanoate, a useful starting material for EPC-syntheses[2,3]. The reproducible enantioselectivity under fermenting conditions in saccharose solution is 93:7 (ca. 85% *ee*), as demonstrated by an *Organic Syntheses* procedure[3]. Under special conditions which can only be realized in a bioreactor (continuous addition of substrate, aeration, etc.), the selectivity can be increased (ca. 96% *ee*)[4]. Obviously, baker's yeast can use several different enzymes for such reductions[5], so that the selectivity depends strongly upon conditions («macroscopic parameters»[3,4,6]), and not only upon the substrate structure[7,8]. Successfully modifying conditions of reductions by baker's yeast is generally a more practical solution for increasing selectivity than is switching to mutants[5] or to other microorganisms[9].

Careful analysis of the published procedures for reductions of β-ketoesters by baker's yeast indicated to us that aerobic conditions[4], the presence of 5–15% ethanol in the medium[4,7], and «ageing» of the yeast[4] might be important for high selectivity, and also that the reaction may be carried out – more economically – in the absence of sugar[10]. In numerous experiments carried out in an Erlenmeyer flask



with indentation shaken at 120 r.p.m., we first replaced glucose as a carbon source in the medium by other «nutrients» such as fructose, (*R*)-lactate, (*S*)-lactate, acetate, glycerol, mannitol, and gluconolactone to find that only with the last mentioned «additive» complete conversion of ethyl acetoacetate (10 g/L in 24 hours) was realized (Table 1). Also, when the substrate ketoester was added one to seven days after «incubation» of the yeast, the selectivity of hydride transfer from the *Re*-face varied drastically from 98:2 to 55:45 (Tables 1 and 2). The optimum conditions – «starving» the yeast for at least four days in 5% aqueous ethanol aerobically – led to a relative activation of the enzyme(s)[5] producing the *S*-enantiomer of 3-hydroxybutanoate (**1**).

Unfortunately, other substrates such as 3-oxopentanoate, 4-chloro-3-oxobutanoate, 4,4,4-trifluoro-3-oxobutanoate, and several α-formyl-esters gave poorer results under «starvation conditions»[12] than under normal conditions[13–15]. Only 2-formylbutanoate was also reduced in good yield (70%) and with high enantioselectivity (95:5) to (*S*)-2-hydroxymethylbutanoate (**2**), the sense of chirality of which was proved by chemical correlation with (*R*)-2-methylbutanoate. The ester **2** is a promising starting material for EPC-syntheses: it has the same features as «Roche» acid [(*S*)-3-hydroxy-2-methylpropionic acid][14,16,17], i.e. enantiotopic functionalized branches, and will provide products with ethyl-substituted chirality centers.

Table 1. Reduction (200 mL distilled H_2O, 25 g yeast) of ethyl acetoacetate (10 g/L) with addition of D-gluconolactone (10 g/L).

Incubation time before substrate feeding [d]	(*S*)-**1**: $[\alpha]_D$ ($CHCl_3$) [°]	*ee* [%][a]
1	+ 36.7	84.2
2	+ 36.7	84.2
3	+ 34.2	78.4
5	+ 27.2	62.4
7	+ 4.2	9.6

[a]) By optical comparison with $[\alpha]_D = 43.6$ ($c = 1$, $CHCl_3$)[11].

Table 2. Reduction (190 mL distilled H_2O, 10 mL ethanol, 25 g yeast) of ethyl acetoacetate (5 g/L).

Incubation time before substrate feeding [d]	(*S*)-**1**: $[\alpha]_D$ ($CHCl_3$) [°]	*ee* [%][a]
1	+ 39.2	89.9
2	+ 40.1	92.0
3	+ 40.3	92.4
4	+ 41.8	95.9
7	+ 41.8	95.9

[a]) See footnote in table 1.

General Procedure

A suspension of 125 g baker's yeast (Klipfel AG, Rheinfelden) in 1000 mL H_2O/EtOH 95:5 was shaken (120 rpm) at 30 °C in a 2-L Erlenmeyer flask with indentation for 4 days. After the addition of the substrate the reaction was followed by GC (Pluronic L 64 column, 20 m, 3 min at 70 °C, then rising by 13 °C/min). After completion (2–3 days) the mixture was centrifuged (20 min, 7000 rpm) and the supernatant was extracted continuously with ether (4 days). The organic layer was dried over $MgSO_4$, filtered, evaporated, and purified by bulb-to-bulb distillation (air-bath temperature in brackets).

Ethyl (S)-3-hydroxybutanoate (**1**): Following the general procedure, 5.0 g (38 mmol) ethyl acetoacetate provided, after distillation (90–100 °C/15 Torr), 3.54 g (70%) of **1** as a colourless liquid. $[\alpha]_D^{RT} = +40.9°$ ($c = 1$, $CHCl_3$), 94% *ee* ([11]: $[\alpha]_D^{RT} = +43.6°$ ($c = 1$, $CHCl_3$), optically pure). – ^{1}H-NMR ($CDCl_3$): 4.15 (q, $J = 7$ Hz, 2 H, $-OCH_2CH_3$), 4.3–4.0 (m, 1 H, H–C(3)), 3.5 (br. s, 1 H, OH), 2.45 (m, 2 H, H–C(2)), 1.25 (t, $J = 7$ Hz, 3 H, $-OCH_2CH_3$), 1.2 (d, $J = 7$ Hz, 3 H, H–C(4)). – GC: retention time 4.61 min.

Ethyl (S)-2-hydroxymethylbutanoate (**2**): Following the general procedure, 3.7 g (26 mmol) ethyl 2-formylbutanoate[18] provided, after distillation (95–105 °C/15 Torr), 3,3 g (88%) of **2** as a colourless liquid. $[\alpha]_D^{RT} = +2.1°$ ($c = 4$, MeOH), 91% *ee* [(*R*)-enantiomer [19]: $[\alpha]_D^{RT} = -2.0°$ ($c = 4$, MeOH), 86.3% *ee*]. – ^{1}H-NMR ($CDCl_3$): 4.2 (q, $J = 7$ Hz, 2 H, $-OCH_2CH_3$), 3.75 (m, 2 H, $-CH_2OH$), 2.5–1.9 (br. m, 2 H, H–C(2) and OH), 1.7 (m, 2 H, H–C(3)), 1.25 (t, $J = Hz$, 3 H, $-OCH_2CH_3$), 0.95 (t, $J = 7$ Hz, 3 H, H–C(4)). – GC: retention time 6.79 min.

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