107. Preparative Asymmetric Reduction of 3-Ketobutyrate and -valerate by Suspended Cells of Thermophilic Bacteria *(Thermoanaerobium brockii)* in Ordinary Laboratory Equipment[1])

by **Dieter Seebach*, Fabio Giovannini**[2]), and **Bernd Lamatsch**[3])

Laboratorium für Organische Chemie der Eidgenössischen Technischen Hochschule, ETH-Zentrum, Universitätstrasse 16, CH–8092 Zürich

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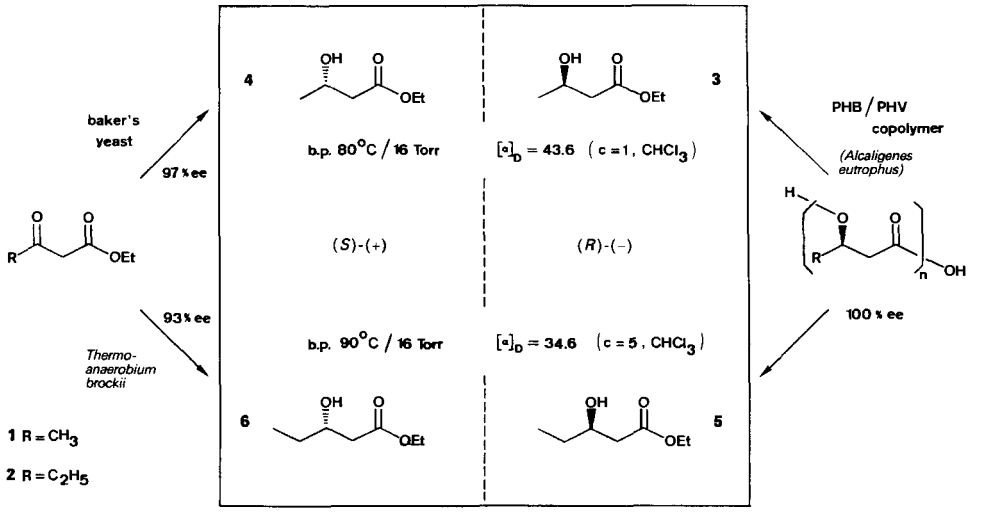
The thermophilic and anaerobic bacteria specified in the title are isolated on a 0.8 kg scale by tangential flow filtration and centrifugation from a 300-l bioreactor. The microorganisms are stored in a freezer (–20°) and used, analogously to baker's yeast, for asymmetric reductions. Thus, ethyl 3-ketovalerate (4.3 g/l (H_2O) is converted in 40% yield to (*S*)-3-hydroxyvalerate (**6**), with an enantiomeric excess of 93% (24 h at 72°).

Yeast reductions of organic substrates are now part of the preparative arsenal of organic synthesis, see *e.g.* the preparation of ethyl (*S*)-3-hydroxybutyrate (**4**) by asymmetric reduction of acetylacetate **1** in a recent *Organic-Syntheses* procedure [4]. The analogous yeast reduction of the 3-ketovalerate **2** proceeds only with poor enantioselectivity [5]. Thus, since the (*R*)-enantiomers **3** and **5** are both readily available, for instance by depolymerization of polyhydroxybutyrate (PHB) and -valerate [6] [7], the (*S*)-hydroxyvalerate **6** is the only member of this series to which we had no ready access as a chiral building block for syntheses.



[1]) For a preliminary communication and for a description of the biotechnological part of this project see [1–3].

[2]) Part of the projected Ph. D. thesis of *F. G.*, ETH Zürich.

[3]) Recipient of a scholarship for undergraduate studies abroad, by the *Studienstiftung des Deutschen Volkes* (1984). On leave from the University of Karlsruhe (T.H.), Germany.

It is known from the work of *Prelog* and his coworkers [8] that the same substrate can be reduced from opposite faces (*Re* or *Si*) by different microorganisms. It is now possible to test hundreds of microorganisms in a relatively short time on an analytical scale [9]. Some time ago, we started a program of scaling up such transformations to see whether microorganisms other than yeast could be handled and employed as easily in normal laboratory equipment, see *e.g.* the production of PHB by *Alcaligenes entrophus H16* [6] [10]. An especially attractive type of microorganisms appeared to be thermophilic bacteria and other bacteria which are able to flourish in harsh environments. At the beginning of our work, nothing was known about the capability of such unusual microorganisms to perform biotransformations of added substrates. Hydride transfer by isolated reductases of *Thermoanaerobium brockii* had been studied by *Zeikus* and his coworkers [11].

In a collaboration with the biotechnology laboratory of the ETH, we first tested for the best growth conditions of these particular thermophiles, and then treated their cultures with different β-ketoester substrates to study their behaviour under different conditions in a bioreactor. The results are described separately [1–3]. At the end of these investigations, we had a larger amount of cells isolated from the bioreactor by tangential filtration and centrifugation: 0.8 kg containing 25% of cells and 75% of H_2O, see *Exper. Part.* Like yeast, the cell mass could be frozen and stored for long periods of time (2 months) at −20°. *Thermoanaerobium brockii* turned out to be quite sturdy. Being an anaerobic microorganism, *it was handled just like air-sensitive organometallic reagents,* and eventually, we reduced 4–5 g of the β-ketoesters **1** and **2** per liter of H_2O with 75 g of cells at 72° under an Ar atmosphere.

In contrast to baker's yeast, *Thermoanaerobium brockii* reduces *both* the butyrate **1** and the valerate **2** to the corresponding hydroxy compounds of (*S*)-configuration (**4** and **6**, resp.) with better than 90% enantiomeric excess (% ee).

Other substrates, such as α-formyl esters, 4,4,4-trifluoro-3-oxobutyrate, and 3-oxohexanoate, which were reduced by *Thermoanaerobium brockii* in the bioreactor [1–3] could not be converted under the conditions described here; the substrates disappeared, but no products could be detected, due either to chemical instability of substrate or product, or to irreversible absorption, or to metabolization.

Experimental Part

1. *General.* Distillations: *Kugelrohr* oven *Büchi GKR-50* (air-bath temp.). Optical rotations: *Perkin-Elmer 241* polarimeter (concentration *c* (g/100 ml) and solv. in brackets). ^{1}H-NMR spectra: *Varian EM 390* (δ's in ppm relative to TMS as internal standard, *J* in Hz). Gaschromatograms: *Carlo Erba Fractovap 4160 HRGC* with a *Pluronic-L64* capillary column (20 m; 70° × 3 min, then 13°/min until 200°). Centrifugations: *DuPont RC 5 Superspeed Refrigerated Centrifuge* (20 min/9000 V/min).

Thermoanaerobium brockii (DSM 1457) was cultivated in a bioreactor [2] [3] (300-l working volume); the cells were harvested first by tangential flow filtration under Ar (0.2 μm membrane, *Arcoflux, Gelman*) until the cell-containing volume was reduced to 5 l and subsequently by centrifugation under Ar. The wet cells (*ca.* 25% cells/75% H_2O) were weighed out at + 4° in 15-g portions, packed in aluminium foil, and stored at −20° (no change after 2 months).

2. *General Procedure for the Resuspension of the* Thermoanaerobium brockii *Cells.* To a 3-necked flask (A) equipped with reflux condenser and magnetic stirring bar were added 200 ml of dist. H_2O, 4.4 g of 2-([2-hydroxy-1,1-bis(hydroxymethyl)ethyl]amino)ethanesulfonic-acid (TES) buffer *(Sigma)*, and 1 g of glucose. The soln. was brought to pH 8 with 4N KOH, and several drops of an aq. resazurin *(Fluka)* soln. were added as indicator. In a second 3-necked flask (B), which had been flushed with Ar, was placed a 15-g portion of frozen *Thermoanaerobium*

brockii cells. A third 3-necked flask (C) contained a freshly prepared 1% aq. $Na_2S_2O_4$ soln. To ensure O_2-free conditions, all 3 flasks were submitted three times to the following procedure: flasks A, C (cooled in an ice/H_2O bath), or B were exposed to medium vacuum until bubbling (A, C) or foaming (B) began, at which time normal pressure in the flasks was restored with Ar[4]). Any residual O_2 in the sugar soln. (flask A) was reduced by careful, dropwise addition of the $Na_2S_2O_4$ soln. (flask C), transferred *via* syringe, until the resazurin indicator changed colour[5]) (pink to yellow). The syringe containing excess $Na_2S_2O_4$ soln. was allowed to remain in the serum stopper of reaction flask A[6]). The sugar soln. (A) was heated to 72°. The cells (flask B) were suspended in a small quantity of the hot sugar soln. and injected into flask A. This was repeated three times ensuring the complete transfer of the cells (all transfers were made *via* syringe).

3. *Reduction of β-Oxoesters.* After the cells had been stirred at 72° for 1 h, the β-oxoester was added. The reaction was followed by gas chromatography, and after completion[7]) the soln. was allowed to cool, centrifuged, and the supernatant was extracted with AcOEt (3 × 200 ml). The combined org. phases were dried ($MgSO_4$), the solv. evaporated at 10 Torr, and the crude product purified by bulb-to-bulb distillation.

4. *Products.* 4.1. (S)-(+)-*Ethyl 3-Hydroxybutyrate* (**4**). Following the general procedure, 1.0 g (7.7 mmol) of ethyl acetylacetate (**1**) provided, after bulb-to-bulb distillation at 10 Torr/100°, 625 mg (62%) of **4** as a colourless liquid. $[\alpha]_D^{r.t.} = +39.9$ ($c = 1.8$, $CHCl_3$), 92% ee ([6]: $[\alpha]_D^{r.t.} = 43.6$ ($c = 1.2$, $CHCl_3$)). ^{1}H-NMR ($CDCl_3$): 4.3–4.0 (*m*, H–C(3)); 4.17 (*q*, $J = 7$, OCH_2CH_3); 3.2 (br. *s*, OH); 2.5–2.4 (*m*, 2H–C(2)); 1.28 (*t*, $J = 7$, OCH_2CH_3); 1.24 (*d*, $J = 7$, 3H–C(4)).

4.2. (S)-(+)-*Ethyl 3-Hydroxyvalerate* (**6**). Following the general procedure, 860 mg (6.0 mmol) of ethyl 3-oxovalerate (**2**) provided, after bulb-to-bulb distillation at 10 Torr/120°, 344 mg (40%) of **6** as a colourless liquid. $[\alpha]_D^{r.t.} = +32.2$ ($c = 5.1$, $CHCl_3$), 93% ee ([7]: $[\alpha]_D^{r.t.} = 34.6$ ($c = 5$, $CHCl_3$)). ^{1}H-NMR ($CDCl_3$): 4.15 (*q*, $J = 7$, OCH_2CH_3); 4.10–3.75 (*m*, H–C(3)); 3.15 (br. *s*, OH); 2.50–2.35 (*m*, 2H–C(2)); 1.70–1.40 (*m*, 2H–C(4)); 1.26 (*t*, $J = 7$, OCH_2CH_3); 0.96 (*t*, $J = 7$, 3H–C(5)).

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[4]) Because of the sensitivity of the bacterial cells to the presence of O_2, a positive pressure of Ar was constantly maintained in reaction vessel A.

[5]) The addition of an excess of the $Na_2S_2O_4$ soln. must be avoided.

[6]) Should the indicator colour change back to pink, the O_2 can be reduced by the further addition of small quantities of the $Na_2S_2O_4$ soln.

[7]) After more than 24 h, the success of the reaction was compromised by O_2 contamination. Therefore, in cases where the reaction is not complete within 24 h, the use of smaller quantities of starting material is recommended over the use of longer reaction times.