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Novel unusual microbial dehalogenation during enantioselective reduction of ethyl 4,4,4-trifluoro acetoacetate with baker's yeast

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Abstract—In the course of investigating microbial syntheses for chiral pharmaceutical intermediates, ethyl 4,4,4-trifluoro acetoacetate (**1**) was submitted to baker's yeast reduction. With the purpose of obtaining the D-carbinol in high enantiopurity, several additives were tested for L-reductase inhibitor activity. Allyl alcohol proved to be not only a suitable additive, but also an inducer for effective defluorination of the substrate.

The baker's yeast reduction of ethyl 4,4,4-trifluoro acetoacetate (**1**) is an established synthetic method for the production of L-carbinol **2** as a precursor of novel fluorinated pharmaceuticals, such as the antidepressant befloxatone.[1–6] Baker's yeast produces **2** in 62% ee, while the respective D-carbinol **ent-2** is accessible by adding the L-reductase inhibitor allyl alcohol to the culture.[7]

In order to obtain the D-isomer **ent-2** in high enantiopurity, the L-reductase inhibitor activities of unsaturated thioethers were compared with that of allyl alcohol. Additionally, a blank experiment without additive was conducted (Table 1). None of these additives showed stereoinduction towards the D-carbinol, with the exception of allyl alcohol.

In the course of investigating the influence of the concentration of allyl alcohol on the enantioselectivity of the baker's yeast reduction, an outstandingly remarkable example for dehalogenation at C-4 was observed.

The presence of 1.0 g/l allyl alcohol afforded **ent-2** in 28% ee with total conversion of **1**, which is somewhat lower than the enantiopurity reported by Davoli et al.[7] Upon addition of 2.0 g/l allyl alcohol the ee increased to 76%. However, **ent-2** was present in the reaction mixture in merely 33%.[13]

The main product formed (62%) was ethyl D-3-hydroxybutanoate (**4**). Remarkably, this defluorination occurs at C-4. Several examples for dehalogenations of β-keto esters are hitherto reported, but they refer to α-halogenated β-keto esters and hereof mostly to 2-iodo species.[8,9] The competing dehalogenation of **1** at C-4 takes place solely on the stage of keto ester **1** and furnishes **3** to give **4**. This is due to a three-center resonance stabilization of an intermediate sp^2-center at C-4 by the carbonyl sp^2-centers. Carbinol **ent-2** is not dehalo-

Table 1. L-Reductase inhibitor activities of additives tested for synthesis of **ent-2**

Entry	Additive	Conc. (g/l)	Isomer	ee	$[\alpha]_D^{20}$ (°)	Yield (%)
1	None	–	L	62	+20.1[a]	36.5
2	Phenyl allyl sulfide	1.0	L	62	+20.1	44.3
3	Phenyl vinyl sulfide	1.0	L	62	+20.1	48.0
4	Allyl alcohol	1.0	D	28	−5.9	39.5
5[b]	Allyl alcohol	2.0	D	76	−16.2	35.3

[a] Pure **2**: +20.1° (Ref. 2).

[b] Substrate **1** was defluorinated to give **4**.

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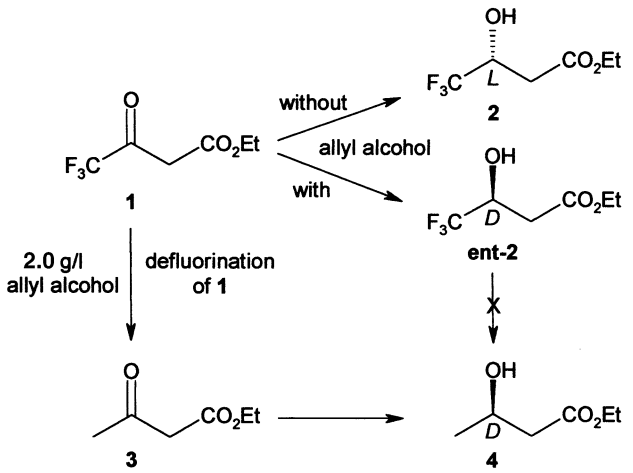

Scheme 1. Concurrence between enantioselective reduction and dehalogenation of **1**.

genated as for these reasons the β-halohydrin C–F bond is not activated. Thus, the dehalo reduction must occur *before* keto reduction (Scheme 1).

This defluorination is especially noteworthy, as reductive dehalogenations of β-keto esters have been observed with activated C–Hal bonds at C-2. Moreover, it is mostly iodo species that are dehalogenated microbially.[8] The enzymic dehalo reductions of 2-halo-β-keto esters have been investigated by several groups who assign these reactions to NADH dependent monoelectronic reduction mechanisms, but the chemistry of these processes is not yet fully understood.[10–12]

To the author's knowledge, this type of reductive microbial dehalogenation of fluorinated β-keto esters has not yet been reported. These results demonstrate impressively, how the effect of additives on the outcome of the microbial enantioselective reductions of halo-β-keto ester **1** is the consequence of a complex interplay of enzymatic activities and competing pathways. The elucidation and understanding of this novel defluorination reaction offers a great potential for dehalogenative microbial transformations and bioremediative processes.

The reasons for this remarkable reaction are currently under investigation.

References

1. Bucciarelli, M.; Forni, A.; Moretti, I.; Prati, F.; Torre, G. *Biocatalysis* **1994**, *9*, 313–320.
2. Seebach, D.; Renaud, P.; Schweizer, W. B.; Zueger, M. F.; Brienne, M.-J. *Helv. Chim. Acta* **1984**, *67*, 1843–1853.
3. Götzö, S. P.; Seebach, D.; Sanglier, J.-J. *Eur. J. Org. Chem.* **1999**, 2533–2544.
4. Antolini, L.; Forni, A.; Davoli, P.; Moretti, I.; Prati, F. *Tetrahedron: Asymmetry* **1998**, *9*, 285–292.
5. Rabasseda, X.; Sorbera, L. A.; Castaner, J. *Drugs Fut.* **1999**, *24*, 1057–1067.
6. Tixidre, A.; Zard, L.; Rossey, G.; Bourbon, A. EP 0736606, 1996; *Chem. Abstr.* **1996**, *125*, 273719n.
7. Davoli, P.; Forni, A.; Moretti, I.; Prati, F.; Torre, G. *Enzyme Microb. Technol.* **1999**, *25*, 149–152.
8. Hamdani, M.; De Jeso, D.; Deleuze, H.; Saux, A.; Maillard, B. *Tetrahedron: Asymmetry* **1993**, *4*, 1233–1263.
9. Cabon, O.; Buisson, D.; Larcheveque, M.; Azerad, R. *Tetrahedron: Asymmetry* **1995**, *6*, 2199–2210.
10. Tanner, D. D.; Stein, A. R. *J. Org. Chem.* **1988**, *53*, 1642–1646.
11. DeCarvalho, M.; Okamoto, M. T.; Moran, P. J. S.; Rodrigues, J. A. R. *Tetrahedron* **1991**, *47*, 2073–2080.
12. Aleixo, L. M.; DeCarvalho, M.; Moran, P. J. S.; Rodrigues, J. A. R. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 1637–1642.
13. **Experimental procedure**:
Materials. Fala baker's yeast was the product of Societé Industrielle de Levure FALA, Strasbourg, France. Ethyl 4,4,4-trifluoro acetoacetate (**1**), additives and organic solvents were purchased from Fluka and Aldrich.
*Baker's yeast reduction of ethyl 4,4,4-trifluoro acetoacetate (**1**)*. The fermentations were carried out in a flask containing 2 L of tap water, 20 mL of **1**, 250 g Fala baker's yeast, and the respective amount of allyl alcohol. After 20 h the products were recovered by extraction with *tert*-butyl methylether, dried over sodium sulfate, and then evaporated to dryness under reduced pressure.
Determination of the extent of conversion. The conversions of the yeast reduction were measured using a J & W Scientific DB-5 column (30 m, 0.25 mm i.d.) at 50°C (isothermal). The pressure of N_2 gas was 80 kPa; the temperatures of the injector and the detector were 210 and 260°C, respectively. The keto-substrate **1** and products **2** and **ent-2** were observed at retention times of 3.7 and 5.5 min, respectively. The % conversions were determined using an integrator.
Determination of enantiomeric excess (ee). After the conversion and rates had been analyzed, the crude mixture was distilled in vacuo at 5 mbar. The purified carbinolic fractions were converted into the corresponding trifluoro acetates by reacting with a 1.2 molar amount of trifluoro acetic acid anhydride in dry CH_2Cl_2 at 65°C. After the reaction was complete, the volatile components were evaporated. GC analysis of the resulting trifluoro acetate was conducted using a Macherey & Nagel Lipodex A column (50 m, 0.25 mm i.d.) running a ramp from 60 to 120°C with 10°C/min. The trifluoro acetates of $NaBH_4$ reduced **1** were used to find the suitable conditions for chiral GC analysis. The pressure of N_2 gas was 130 kPa; the temperatures of the injector and the detector were 250 and 260°C, respectively. The D-carbinol **ent-2** was observed at a retention time of 9.1 min and the L-enantiomer **2** at 10.2 min. The relative amounts were determined using an integrator.
Determination of absolute configuration. The absolute configuration of the products was determined using a Perkin–Elmer polarimeter 341 ($c=1.0$, $CHCl_3$) and comparison of the obtained results with reference data in Ref. 2.