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PREPARATION OF BOTH ENANTIOMERS OF ETHYL 4,4,4-TRIFLUORO-3-HYDROXY BUTANOATE BY ENANTIOSELECTIVE MICROBIAL REDUCTION

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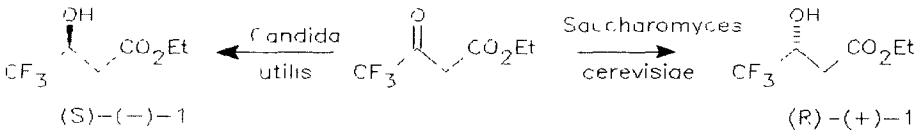
Abstract. The effect of some parameters, i.e. temperature, time, pH and concentration, on the baker's yeast reduction of ethyl 4,4,4-trifluoroacetoacetate is presented. The enantiomeric excess of the R enantiomer appeared to increase up to 76% when the temperature of the reduction decreased. The other factors do not appear to improve the enantioselectivity of the reaction. Reduction with *Candida utilis* allowed preparation of the S enantiomer in higher optical purity than previously reported.

The use of baker's yeast *Saccaromyces cerevisiae* in enantioselective transformations is an useful and widely utilized process for the preparation of chiral alcohols[1]. However, although reduction of ß-keto esters has been well documented[1,2], the corresponding reduction of ethyl 4,4,4-trifluoroacetoacetate has been scarcely reported[3,4]. Moreover, the relatively modest enantiomeric purity (45-53% enantiomeric excess) of the reduction product, ethyl (R)-(+)-4,4,4-trifluoro-3-hydroxy butanoate 1, induced us to study the effect of some parameters to improve the enantioselectivity of the reaction, i.e. temperature, pH, time,... as well as to develop a direct, yeast-mediated preparation of the S enantiomer. The synthesis of this compound has been based so far only on the biotransformation of the racemic alcohol 1 or its acetate with the archaebacterium *Halobacterium halobium*[5a] or with immobilized lipase P[5b], respectively, in very low ee (11-20%). Only one preparation from ethyl 4,4,4-trifluoroacetoacetate with continuously growing cultures of *Thermoanaerobium brockii*[5c] at 72ºC has been reported, also in low enantiomeric purity (24% ee). Our interest in the development of fluorinated analogues of insect sex pheromones[6], and the requirement of fluorinated chiral compounds in a on-going project of our laboratory make the goals outlined above highly desirable.

In preliminary experiments carried out with powder baker's yeast, reduction of ethyl 4,4,4-trifluoroacetoacetate afforded in our hands the expected R enantiomer in similar yields (50-70%) and ee (45-55%) as previously described[3]. We have found that by *lowering* the temperature the ee of (R)-1 noticeably *increased*, being 76% the maximum ee value obtained when the reduction was done at 7ºC (entry 8, table 1). Lower temperature (2ºC, entries 9-10) did not further improve the enantioselectivity of the reaction, whereas higher temperatures than 34ºC led to inferior results (entry 1, table 1). In the common range of 26-34ºC the effect was practically null. Although some reports have been found in the literature about the temperature sensitive synthesis of fatty acids and transfer RNAs in vivo in *S. cerevisiae*[7], to our knowledge and very surprisingly, this is the first example of a temperature dependent enantioselective reduction with baker's yeast reported. It is likely

that low temperatures may, at least partially, render kinetically unfavourable the action of the *L*-enzyme(s) of the yeast responsible for the **S** enantiomer production[8a]. Effect of the reaction time (entries 3-4 and 9-10, table 1) both on the yield and ee of **(R)-1** was practically insignificant. Reduction of the keto ester with other types of baker's yeast (type I and type II from Sigma Chem. Co.) (temp. 34ºC, [yeast]=0.05, yeast/substrate=5)



required longer reaction times than with the powder form but afforded **1** in similar chemical and optical yields.

The effect of pH on the reduction was studied by using standard buffer solutions at pH=3-9 (table 2). The results showed in most cases a marked decrease in the enantiomeric purity of the product, presumably due to a difference in inhibition of the **R** and **S** isomer producing enzymes caused by the addition of the buffer solution salts, as it has been previously noted[8b]. The boric acid/KCl/NaOH mixture, constituents of the pH 9 buffer solution, was probably innocuous towards the enzyme system since it afforded the normal value of ee expected for the transformation temperature (36ºC).

Table 1. Effect of temperature and time on the reduction of 4,4,4-ethyl trifluoroacetoacetate with powder baker's yeast[a,b]

Entry	Yeast/Substrate	Temp(ºC)	Time(h)	Yield	ee	Enantiomer
1	13	38	48	69	35	R
2	13	34	30	71	44	R
3	13	30	24	47	45	R
4	13	30	2	50	42	R
5	13	26	42	70	44	R
6	13	22	48	71	49	R
7	13	15	24	75	68	R
8	13	7	48	45	76	R
9	13	2	72	67	69	R
10	10	2	144	54	74	R

[a] [baker's yeast]=0.1 g/ml.
[b] The ee have been calculated in base to the specific rotation values in comparison with the previously reported[3d] or by the (S)-MTPA ester (Mosher's method[9]). Attempts to use ^{1}H NMR chiral shift reagents proved to be unsuccessful in this case.

Concentration of the yeast did not appear to have a remarkable effect neither on the yield nor on the ee of **1** (table 3). Lower amount of glucose (entry 3) afforded lower ee whereas longer reaction time (entry 5) caused better recovery of the reduction product (see below).

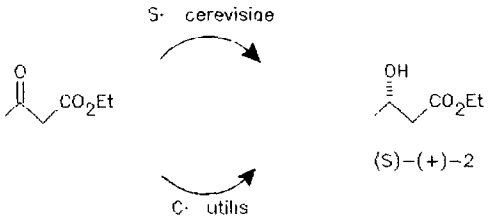


Table 2. Effect of pH on the baker's yeast reduction of ethyl 4,4,4-trifluoroacetoacetate in the presence of a buffer solution[a].

Initial pH	Yeast/substrate	Yield	ee	Enantiomer
3	5	66	19	R
5	5	71	12	R
7	5	64	23	R
9	5	41	38	R

[a][Yeast]=0.05 g/ml.

Table 3. Effect of concentration on the baker's yeast reduction of ethyl 4,4,4-trifluoroacetoacetate (36ºC).

Entry	Yeast/substrate	Glucose/yeast	Time(h)	Yield	ee	Enantiomer
1	5	1	24	44	36	R
2	10	1	48	67	31	R
3	10	0.5	48	56	22	R
4	20	1	24	63	37	R
5	5	1	72	72	30	R

When baker's yeast was replaced by the commercially available *Candida utilis* (Sigma Chem. Co.) the (S)-(-)-1 enantiomer was obtained in moderate to good yields (34-98%) and higher enantiomeric purity (up to 59%) than previously described[5]. The effect of temperature and time was studied and the results outlined in table 4. In this case, longer reaction times than with baker's yeast were required, and the enantioselectivity of the process *increased* with the temperature, being 38ºC the optimum temperature found. It is interesting to note that, although most reactions were completed after 3-5 days, when the transformations were extended up to 7 days the chemical yields became higher, apparently due to a more effective extraction of the product after long periods of time. The maximum temperature of utilization of the yeast appeared to be lower than 42ºC.

The eventual ability of the *C. utilis* enzyme system of preparing different enantiomers than baker's yeast was tested when both yeasts were allowed to react with ethyl acetoacetate. Unfortunately, whereas the latter gave the expected (S)-(+)-2 in good yields (80-83%) and ee (76-91%), the former disappointingly afforded the same enantiomer in 44% yield and only 22% ee after 7 days reaction. Presumably, the *L*-enzyme of the *C. utilis* system favors affinity for the trifluoromethylated ester whereas the *D*-enzyme preferably reduces the parent non-fluorinated molecule. In any case, the possibility of obtaining the S enantiomer directly

from the keto ester in very high ee by fractional crystallization from the racemate, as it has been done by the R enantiomer,[3a] makes this process attractive from a synthetic viewpoint.

Table 4. Effect of temperature and time on the reduction of 4,4,4-ethyl trifluoroacetoacetate with *Candida utilis*[a,b].

Entry	Yeast/substrate	Temp	Time(d)	Yield	ee	Enantiomer
1	5	28	7	80	48	S
2	5	28	5	54	29	S
3	13	30	5	81	31	S
4	13	34	5	98	35	S
5	13	38	5	38	46	S
6	5	38	3	34	59	S
7	5	38	7	63	57	S
8	5	42	7	--	--	-
9	13	42	7	--	--	-

[a][Yeast]=0.05-1 g/ml.
[b]The ee have been calculated by the (S)-MTPA ester (Mosher's method[9]).

In summary, enantioselective preparation of both enantiomers of ethyl 4,4,4-trifluoro-3-hydroxy butanoate by reduction of ethyl 4,4,4-trifluoroacetoacetate with baker's yeast and *C. utilis* has been achieved in good yields and ee. The temperature has been found to be an important factor to consider in enzymatic reduction processes.

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