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Baker's Yeast Reduction of β-Keto Esters in Petrol

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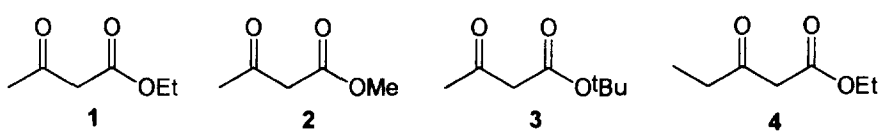
Abstract: *A none fermenting suspension of Baker's yeast in petrol containing a small amount of water reduces a variety of β-keto esters to the corresponding optically active β-hydroxy esters.*

Homochiral β-hydroxy esters are valuable starting materials for the asymmetric synthesis of a variety of chiral products.[1] Optically active β-hydroxy esters are usually prepared by the asymmetric reduction of β-keto esters using either a synthetic chiral reducing agent[2] (or chiral catalyst with achiral reducing agent), or by using an oxido-reductase enzyme, often as intact yeast cells.[3] The latter process is particularly attractive in terms of cost, however the traditional way of carrying out yeast reductions uses a fermenting solution of the yeast in an aqueous solvent system.[3] This can cause synthetic problems, particularly with large scale reactions due to the relatively large quantities of solvent required, the hydrophobic nature of some β-keto esters, and the hydrophilic nature of many β-hydroxy esters. Recently however, Smallridge *et al.* reported that a none fermenting suspension of Baker's yeast in an organic solvent (optimally petrol) was capable of reducing ethyl acetoacetate **1**, giving ethyl 3-hydroxybutanoate with similar enantiomeric excess and yield to those obtained using fermenting Baker's yeast.[4] Additionally, Seebach *et al.* have reported the use of a mixture of isopropyl hexadecanoate and soybean phospholipids as the reaction medium for the reduction of β-keto-esters using Baker's yeast.[5] In this paper, we report the reduction of a variety of β-keto esters by Baker's yeast in petrol, and show that in some cases the results contrast with those obtained in aqueous solution.



1 **2** **3** **4** **5** **6** **7**

For all of the work described in this paper, we used freeze dried Baker's yeast (*Saccharomyces cerevisiae*) as supplied by Sigma. As this yeast is from a different supplier and so may have different activity to

that reported by Smallridge *et al.*, the efficiency of the yeast in reducing ethyl acetoacetate was first investigated, the results being shown in Table 1. Previous work[4] had already shown, that some water must be present in the petrol solution for any reduction to occur. Thus the amount of yeast, water, and petrol (fraction boiling between 40 and 60°C) were all varied, whilst the reaction time was kept constant at 18 hours to determine the minimum quantities of each necessary for complete reduction of ethyl acetoacetate. After the reaction mixture had been mechanically stirred for 18 hours, the petrol was decanted from the yeast and the latter extracted with 3x30ml of ethyl acetate. The combined organic layers were dried with magnesium sulphate, evaporated *in vacuo*, and the residue purified by bulb to bulb distillation, the products were then analysed by GC, nmr, and polarimetry. As the results in Table 1 show, it was advantageous to use larger quantities of yeast and water to those previously reported in order to obtain complete reduction of ethyl acetoacetate (entry 7). This is probably due to the yeast being from a different supplier, and in particular it should be noted that under these none fermenting conditions, the limiting factor will be the amount of reduced cofactor present in the yeast cells, as there is no mechanism for the regeneration of cofactor, except possibly reoxidation of the β-hydroxyester. Under the optimum conditions (entry 7), ethyl (*S*)-3-hydroxybuyrate was obtained with excellent enantiomeric excess, and respectable yield though the latter was slightly lower than that reported by Smallridge *et al.*, (58%) presumably due to absorption of the starting material and/or product within the larger quantity of yeast cells. For comparison, under aqueous conditions the β-hydroxyester is formed with >96% enantiomeric excess in 32-56% yield.[3]

Table 1: Reduction of Ethyl Acetoacetate by Baker's Yeast[a]

Entry	Yeast (g)	1 (g)	Water (ml)	Yield (%)	Conversion (%)	ee (%)[c]
1	7.5	1	6	49	45	>98[6] (*S*)
2	7.5	2	6	40	40	>98 (*S*)
3	7.5	3	6	30	40	>98 (*S*)
4	7.5	4	6	25	10	>98 (*S*)
5	7.5	5	6	16	33	36 (*S*)
6	15	2	24	30	100	>98 (*S*)
7	22.5	2	18	38	100	>98 (*S*)
8	22.5	2	15	40	50	>98 (*S*)
9	30	2	24	39	100	>98 (*S*)
10[b]	22.5	2	18	12	100	87 (*S*)

a) All reactions were carried out at least in duplicate, and except entry 10) used 250ml of petrol. Recovery refers to the total amount of recovered material (starting material and/or product). Conversion is the % product in the recovered material. b) Using 125ml petrol. c) Enantiomeric excesses are based upon the specific rotation of the isolated material, after correction for the amount of alcohol present. A value of >98% means that the specific rotation matched that in the literature.

Having determined the optimal conditions for the reduction of ethyl acetoacetate, the effect of varying the ester function was investigated using methyl ester **2** and *t*-butyl ester **3** respectively, the results being presented in **Table 2**. Under the optimal conditions for the reduction of ethyl acetoacetate, the methyl ester **2**, gave the corresponding optically pure alcohol as the only product, though in a reduced 15% yield (entry 1). A low yield of this β-hydroxy ester (with 87% ee) has also been reported using Baker's yeast under aqueous conditions.[3] In the case of methyl ester **2**, doubling the amount of yeast (entry 2) resulted in a further reduction in the chemical yield. This effect is presumably due to the absorption of the starting material or product within the yeast cells as

is observed for the corresponding ethyl ester **1**. Thus the quantity of yeast and water were halved (entry 3) in the hope that this would increase the chemical yield, whilst not adversely affecting the optical yield. This proved to be partially successful, as 40% of the material was recovered, though only 45% of this had been converted to optically pure alcohol. In the case of t-butyl ester **3**, the standard conditions (entry 4) gave a good yield of a 1:1 mixture of starting material and optically pure alcohol. Increasing the amount of yeast used (entry 5,6) increased the conversion to alcohol, though at the expense of the chemical yield.

Next, the effect of lengthening the alkyl chain was investigated using derivatives **4-6**. The results in Table 2 (entry 7-11) show that β-keto esters **4** and **5** are reduced by yeast under these conditions. Compound **4** however, gives ethyl (*S*)-3-hydroxypentanoate with relatively low ee, whereas compound **5** gives ethyl (*S*)-3-hydroxyhexanoate with excellent ee, though it was not possible to drive this reduction to completion. By comparison, under aqueous conditions Baker's yeast reduction of compound **4** gives the (*R*)-enantiomer of the β-hydroxy ester in 67% yield with 40% ee.[3] However, ethyl benzoylacetate **6** was not reduced at all either under the optimal conditions for the reduction of ethyl acetoacetate (entry 12), or using double the amounts of water and yeast (entry 13). Interestingly, compound **6** has been reported to be reduced by Baker's yeast under aqueous conditions.[7] It appears therefore that the failure of compound **6** to react in petrol is due to the hydrophobicity of this compound preventing it from entering the aqueous environment around and/or within the yeast cells rather than to the compound being too big to fit into the active site of the oxidoreductase enzyme.

Table 2: Reduction of β-Keto Esters by Baker's Yeast[a]

Entry	Substrate	Yeast (g)	Water (ml)	Recovery (%)	Conversion %	ee[b]
1	**2**	22.5	18	15	100	>98[8] (*S*)
2	**2**	45	35	2.5	100	>98 (*S*)
3	**2**	11	9	40	45	>98 (*S*)
4	**3**	22.5	18	60	50	>98[5] (*S*)
5	**3**	45	35	39	66	>98 (*S*)
6	**3**	60	40	28	75	>98 (*S*)
7	**4**	22.5	18	32	66	47[9] (*S*)
8	**4**	45	36	25	100	45 (*S*)
9	**5**	22.5	18	45	14	>98[10] (*S*)
10	**5**	45	35	45	55	>98 (*S*)
11	**5**	11	9	69	10	>98 (*S*)
12	**6**	22.5	18	32	0	0
13	**6**	45	35	0	0	0
14	**7**	22.5	18	45	50	>98[7]
15	**7**	45	35	42	86	>98
16	**7**	60	40	30	86	>98

Footnotes as for Table 1.

Finally, the effect of introducing a group into the 2-position of the β-keto ester was investigated using ethyl 2-methyl acetoacetate **7**. Compound **7** is expected to undergo racemisation under the reaction conditions, whilst the product β-hydroxy ester is expected to be configurationally stable. Thus in principle, enantiomerically and diastereomerically pure β-hydroxyester could be obtained by the yeast reduction of compound **7**. In practice, compound **7** was found to be a good substrate for reduction by Baker's yeast under these conditions, the optimum conditions being entry 15. The β-hydroxy ester was formed as a 4:1 ratio of diastereomers, and

comparison of the nmr spectra and specific rotations with those reported in the literature[11] showed that the (2*R*, 3*S*) diastereomer was the major product, as it is during the reduction of ethyl 2-methylacetoacetate in aqueous solvents.[7]

In summary, we have shown that a variety of β-keto esters can be stereospecifically reduced by Baker's yeast in petrol, and that in one case this changes the enantioselectivity of the reduction. The substrate specificity of the reaction also differs between petrol and water. The present procedure is considerably more convenient than the previous methodologies which utilise aqueous solvents.

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