The Use of Organic Solvent Systems in the Yeast Mediated Reduction of Ethyl Acetoacetate

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(Received March 29, 1994)

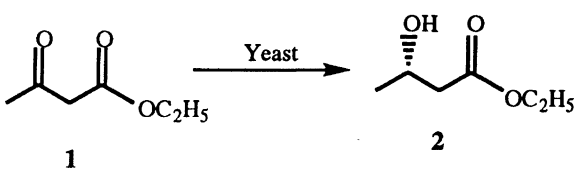
The reduction of ethyl acetoacetate to ethyl (*S*)-3-hydroxybutyrate, mediated by freeze-dried yeast, proceeds in good yield (53—58%) and with high enantioselectivity (>96%ee) in a number of organic solvents; petroleum ether, diethyl ether, toluene, and carbon tetrachloride. A small amount of water (0.8 ml (g-yeast)$^{-1}$) is required for the reaction to proceed. The water/yeast/substrate ratio and the solvent polarity have been found to significantly influence the reactivity of the system. The enantiomeric excess was determined by gas chromatography employing a chiral column.

Yeast is often employed as a reducing agent in organic synthesis, indeed the yeast mediated reduction of β-keto esters is one of the oldest uses of microbes in synthesis.[1] One of the major drawbacks associated with yeast mediated reduction reactions has been the necessity for an aqueous reaction medium which restricts the usefulness of the system for water insoluble compounds or compounds containing water labile functionality. A number of reports have appeared recently which indicate that the reducing capability of yeast is preserved in an organic solvent if the yeast is first immobilized.[2—4] Thus α- and β-keto esters can be reduced in hexane, utilising yeast which has been immobilized in polyurethane or calcium alginate beads. More recently it has been reported that free (unimmobilized) yeast is capable of the reduction of α-keto esters in benzene[5,6] and β-keto esters in petroleum ether.[7] The present work extends the investigation of the yeast induced reduction of a β-keto ester (ethyl acetoacetate) in petroleum ether to a range of polar and nonpolar solvents.

Results and Discussion

Freeze-dried bakers' yeast (Mauri Foods Ltd., Australia) was used for this work. The yeast consists of finely divided particles, hence it is not necessary to grind it to a fine powder to maximize the surface contact area. The reduction of ethyl acetoacetate (Scheme 1) was chosen for this study as both starting material and product are readily separable using gas chromatography and the product, ethyl (*S*)-3-hydroxybutyrate (**2**), has been used in the preparation of a number of natural products.[8]

Effect of Water on Reactivity. No reaction occurred when ethyl acetoacetate was stirred in petroleum ether in the presence of yeast (1 g mmol^{-1}) at room temperature for 24 h. Addition of small amounts of distilled water (0.2—1.2 ml (g-yeast)$^{-1}$) to the reaction system resulted in increasing reduction with complete consumption of starting material occurring with 0.8 ml-water/g-yeast. A similar effect was observed when the reaction was carried out in toluene, diethyl ether and carbon tetrachloride although in these cases complete reduction was not obtained (Fig. 1).



1 **2**

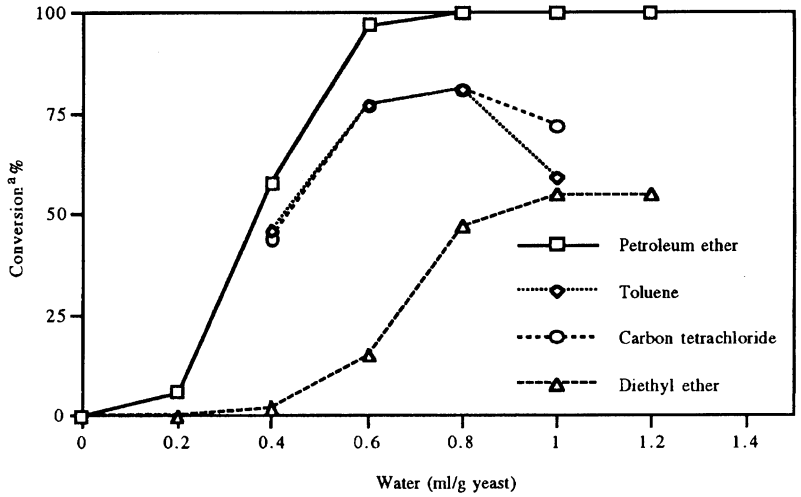
Scheme 1.

It has been demonstrated that the yeast mediated reduction of β-keto esters in aqueous solutions is sensitive to the pH of the reaction medium.[9] Replacing the distilled water with an equivalent amount of buffer (pH=5.0, 6.0, 7.0, 8.0) had no significant affect on the extent of reaction. In all further studies distilled water was used.

A series of reactions with varying water/solvent/yeast ratios established that it is the water/yeast ratio which directly affects the reaction yield (Table 1). Virtually identical conversions were obtained from the yeast-catalyzed reduction of ethyl acetoacetate in petroleum ether with a given water/yeast ratio irrespective of the water/solvent ratio. No reaction occurred when the reaction was conducted in a range of other solvents (acetone, acetonitrile, chloroform, dichloromethane, *N*,*N*-dimethylformamide, ethanol, ethyl acetate, ethyl methyl ketone, isobutyl methyl ketone, and tetrahydrofuran) irrespective of the amount of water added to the reaction system.

From these results it is obvious that both the nature of the organic solvent and the water/yeast ratio affect the reaction. Recently it has been shown that an enzyme becomes fully hydrated when surrounded by a few layers of water molecules.[10,11] It is thought that this hydration layer acts as a microreactor for the enzyme and protects it from any detrimental effects of the bulk organic solvent. It therefore appears that 0.8 ml-water/g-yeast is the minimum amount of water required to form this protective layer around the yeast enzymes for the reduction of ethyl acetoacetate to proceed.

The extent of interaction between the organic solvent and the essential water surrounding an enzyme will



[a] calculated from GC ratio of starting material to product.

Fig. 1. Effect of water quantity on the yeast mediated reduction of ethyl acetoacetate. (1 g-yeast, 1 mmol ethyl acetoacetate, 50 ml solvent).

Table 1. Reduction of Ethyl Acetoacetate in Petroleum Ether with Varying Water/solvent/Yeast Ratios

Water/Yeast	Water/Solvent	Conversion[a)]
ml/g	ml/100 ml	%
0.8	0.8	100
0.8	1.6	100
0.4	0.4	54
0.4	0.8	58
0.4	1.6	58

a) Calculated from GC ratio of starting material to product.

depend on the nature of the solvent. Hydrophilic solvents will tend to remove or distort this protective water layer and cause inactivation of the enzyme, whilst hydrophobic solvents are less able to interfere with the water and thus are expected to be the most suitable nonaqueous media for enzymic reactions.

The partition coefficient of a solvent between water and octanol has been proposed as a measure of the polarity of the solvent.[12)] The logarithm of the partition coefficient ($\log P$) has been shown to correlate reasonably well with the reactivity of a variety of enzymes in organic solvents.[13,14)] As expected, enzymes are more active in nonpolar solvents ($\log P > 2$) and the results from the yeast mediated reduction of ethyl acetoacetate in a variety of solvents follow this trend (Table 2) with the reaction proceeding smoothly in the nonpolar solvents petroleum ether, toluene, and carbon tetrachloride while no reaction occurs in solvents of higher polarity. The activity of yeast in diethyl ether is rather surprising given the low $\log P$ value of this solvent. The higher than expected activity of enzymes in diethyl ether has been previously noted and a $\log P$ correction

Table 2. $\log P$ Values of Solvents Used in Yeast Mediated Reduction of Ethyl Acetoacetate

Solvent	$\log P$[a)]
Petroleum ether	3.5
Toluene	2.5
Carbon Tetrachloride	n/a
Chloroform	2.0
Diethyl ether	0.85
Ethyl acetate	0.68
Tetrahydrofuran	0.49
Acetone	−0.23
Ethanol	−0.24
Acetonitrile	−0.33
Dichloromethane	−0.5
N,*N*-Dimethylformamide	−1.0

a) $\log P$ values obtained from Refs. 12 and 13.

factor proposed to account for the observed activity.[12)]

Effect of Yeast/Substrate Ratio. When the reduction is performed using petroleum ether as the solvent, 1 g of freeze-dried bakers' yeast is sufficient for complete reduction of 1 mmol of ethyl acetoacetate, whilst starting material was still present when the reaction was carried out in toluene, diethyl ether, and carbon tetrachloride. Longer reaction times gave no increase in yield. Increasing the amount of yeast to 2 g, while maintaining the water level at the optimum value of 0.8 ml-water/g-yeast, gave a significantly improved yield in the case of diethyl ether and virtually complete reduction in toluene and carbon tetrachloride (Table 3). These are considered to be the optimum conditions for the yeast mediated reduction of ethyl acetoacetate in these solvents. The yeast-catalyzed reduction of carbonyl groups involves the concomitant oxidation of the coenzyme NAD(P)H to $NAD(P)^{\oplus}$. In aqueous systems various metabolic pathways within the yeast continu-

Table 3. Reduction of Ethyl Acetoacetate in a Variety of Solvents with Differing Amounts of Yeast. (1 mmol Ethyl Acetoacetate, 50 ml Solvent, 0.8 ml-Water/g-Yeast)

Solvent	Conversion/%	
	1 g-Yeast	2 g-Yeast
Petroleum ether	100	a)
Toluene	81	94
Carbon tetrachloride	81	100
Diethyl ether	47	90

a) Not attempted since complete reduction was obtained with 1 g yeast.

ously recycle the NAD(P)$^{\oplus}$ back to NAD(P)H ensuring a continuous supply of the coenzyme. In organic solvents however, regeneration of the coenzymes cannot occur and the extent of reduction is limited by the amount of available NAD(P)H in the yeast. The yeast is therefore providing both the catalyst (oxidoreductase enzyme(s)) and the reagent (NAD(P)H) for the reaction. The increase in the extent of reduction observed when the amount of yeast is doubled (Table 3) can be ascribed to a corresponding increase in the amount of available NAD(P)H.

Yield. Reduction of 1 g of ethyl acetoacetate in petroleum ether, toluene, carbon tetrachloride, and diethyl ether using the previously determined optimum conditions resulted in the isolation of ethyl (*S*)-3-hydroxybutyrate in reasonable yield and high enantiomeric excess (Table 4). It is unclear why the product is isolated in only moderate yield (53—58%) when gas chromatographic analysis of the reaction mixture indicates only product and traces of starting material. It appears likely that some of the starting material and/or product remains in some way bound to the yeast. It is unlikely that the material is physically trapped in the yeast as numerous washings with a variety of different solvents failed to increase the product recovery. It seems likely that chemical binding is involved.

Enantioselectivity. In aqueous solvents the yeast-catalyzed reduction of ethyl acetoacetate results in the formation of ethyl (*S*)-3-hydroxybutyrate[8] although it is possible to obtain the (*R*)-enantiomer by varying the reaction conditions, employing various additives or modifying the yeast.[15] The yeast mediated reduction of ethyl acetoacetate in organic solvent systems also results in the virtually exclusive formation (>96%ee) of ethyl (*S*)-3-hydroxybutyrate under all of the reaction conditions employed so far. This contrasts with the results obtained by Nakamura et al.[6] where the yeast mediated reduction of α-keto esters in water gave the (*S*)-enantiomer whilst in organic solvents the (*R*)-enantiomer is obtained. In the present work, identical ee's were obtained on the crude reaction mixture and the isolated product indicating that selective removal or decomposition of one of the enantiomers had not occurred during workup. The enantiomeric excess was determined by gas chromatographic analysis of the trifluoroacetyl derivative of the ethyl 3-hydroxybutyrate using a chiral GC column (Fig. 2). This work complements the work involving the yeast mediated reduction of α-keto esters in organic solvents[6] although higher isolated yields and higher ee's have been obtained with the β-keto esters. Whilst the reduction of ethyl acetoacetate in organic solvents gives similar yields and ee's to that obtained in water the use of organic solvents involves a considerably simpler reaction system and more straightforward work up procedure.

Table 4. Reduction of Ethyl Acetoacetate in a Variety of Solvents. (1 g, 7.7 mmol Ethyl Acetoacetate, 250 ml Solvent, 0.8 ml-Water/g-Yeast)

Solvent	Yield[a](%)	ee[b](%)
Petroleum ether[c]	58	98
Toluene[d]	53	98
Carbon tetrachloride[d]	56	98
Diethyl ether[d]	58	96

a) Isolated yield. b) Calculated from GC data obtained from the trifluoroacetyl derivative using a chiral column. c) 1 g-yeast/mmol substrate. d) 2 g-yeast/mmol substrate.

Experimental

Instruments. Preparative radial chromatography was performed on a Harrison Research 7924T Chromatotron with glass plates coated with 2 mm thickness layer of Merck Silica gel 60PF/UV_{254}. Gas chromatography was performed on a Hewlett Packard 5890 Series II model with Hewlett Packard 5971 A mass selective detector. The column was an HP-1 (12 m×0.22 mm) with a thickness of 0.33 μm and a

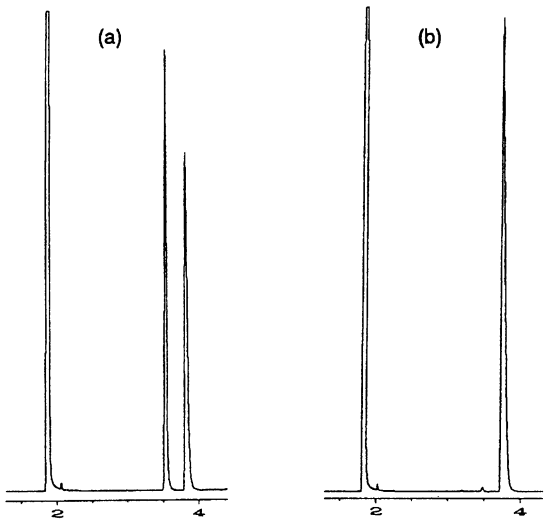


Fig. 2. Gas Chromatography of: (a) racemic mixture of ethyl 3-hydroxybutyrate, (b) ethyl(*S*)-3-hydroxybutyrate isolated from yeast-catalyzed reduction of ethyl acetoacetate in petroleum ether.

nonpolar, crosslinked, methylsiloxane phase. Enantiomeric excess was determined by gas chromatographic analysis on a model 3700 Varian gas chromatograph using a Chiraldex G-TA (30 m×0.25 mm) column. Bulb to bulb distillations were performed on a Buchi GKR-50.

Materials. Freeze-dried bakers' yeast (*Saccharomyces Cerevisiae*, brand name "Tandaco", Mauri Foods Ltd., Australia) was purchased from a local supermarket and stored at room temperature. Ethyl acetoacetate, ethyl 3-hydroxybutyrate, and trifluoroacetic anhydride were purchased from Aldrich Chemical Co.

General Procedure for the Reduction of Ethyl Acetoacetate. In a 500 ml round-bottom flask was placed ethyl acetoacetate (1.00 g, 7.5 mmol), 250 ml of petroleum ether (boiling range 40—60 °C), 6 ml of water and 7.5 g of yeast and the reaction stirred at room temperature. After 24 h, gas chromatography indicated there was only product present in the reaction mixture. The reaction mixture was filtered and the yeast was washed with ethyl acetate (30 ml×3), filtered and the solvent removed under reduced pressure. The product was purified using preparative radial chromatography (petroleum ether : diethyl ether 1 : 4). Bulb to bulb distillation gave ethyl 3-(*S*)-hydroxybutyrate. (0.58 g, 58% yield), (ee=98%). The enantiomeric purity was determined using the method described below. The NMR spectra and other physical data were identical with an authentic sample.

Determination of Enantiomeric Purity. To approximately 1 mg of ethyl 3-hydroxybutyrate was added 0.2 ml of dichloromethane and 0.1 ml trifluoroacetic anhydride in a capped vial which was kept at room temperature for 30 min and then evaporated to near dryness. The residue was dissolved in 0.2 ml ethanol and used to determine the optical purity by gas chromatography. $S:R=99:1$, 98%ee.

The authors wish to thank Mauri Foods Ltd. for providing yeast samples and assistance with technical support. This work was in part supported by an ARC Small Grant.

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