Yeast-Mediated Reduction of *N*-Substituted Acetoacetamides. Improvement of Conversion by Immobilization

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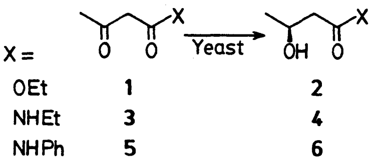
Synopsis. *N*-Ethylacetoacetamide and acetoacetanilide were enantioselectively converted into the corresponding (*S*)-3-hydroxy compounds by alginate- or polyurethane-immobilized bakers' yeasts, while without immobilization the yield of the yeast-mediated reaction was very low.

Microbial transformations of organic molecules are useful for the preparation of chiral compounds. One of the most intensively studied reactions is bakers' yeast-mediated conversion of β-keto esters into the corresponding optically active 3-hydroxy compounds. While ethyl acetoacetate (**1**) is reduced by bakers' yeast to afford ethyl (*S*)-3-hydroxybutanoate (**2**) possessing high optical purity,[1)] chemical and optical yields of reactions of analogs of **1** fluctuate widely. Improvement of stereoselectivity of the microbial reduction of the analogs of **1** by chemical modification of the substrates[2)] and stereochemical control of the reaction by the use of immobilized yeast[3,4)] were described. In this note we will report microbial reduction of *N*-substituted acetoacetamides into the corresponding alcohols and marked enhancement of the conversion by immobilizing bakers' yeast.



Bakers' yeast is commercially available in the form either of dried granules or of freshly prepared cake. The former is more convenient since it is stable and can be stored without significant deterioration. Microbial reduction of *N*-ethylacetoacetamide (**3**), a nitrogen analog of **1** was attempted using granular bakers' yeast. Under the conditions which assured efficient conversion of **1** to **2**, however, the amide **3** failed to give the corresponding alcohol, *N*-ethyl-3-hydroxybutanamide (**4**). Acetoacetanilide (**5**) was then subject to examination as a substrate of yeast-mediated reduction because of the ease of detection by TLC and separation of the expected reduction product, 3-hydroxybutananilide (**6**), as well as the unchanged **5**. Incubation of **5** for 7 days with fermenting yeast derived from granular dry yeast did not give the reduction product **6** but resulted in the recovery of **5** in 90% yield. When fresh yeast was used instead of dry yeast, however, the hydroxy derivative **6** was obtained in 11% yield. Brewers' yeast was also able to reduce **5** though in low yield (2%). Cultivated suspension of a strain, *Saccharomyces cerevisiae* H-194, was found to convert **5** into **6** in higher yield (48%) as given in Table 1.

The results described above leads to the following suggestion. The apparent inability of the dry bakers'

Table 1. Chemical Yield and Optical Purity of Yeast-Mediated Reduction of *N*-Substituted Acetoacetamides

Method of microbial reduction	Yield of reduction product (%)	e.e. of product estimated by ^{1}H NMR (%)	Recovery of unchanged substrate (%)
Transformation of **3** to **4**			
bakers' yeast (dry), suspension	0		82
bakers' yeast (fresh), suspension	0		90
S. cerevisiae H-194, growing culture	0		90
bakers' yeast (dry), immobilized by alginate	9	90—95	54
brewers' yeast, immobilized by alginate	8		51
bakers' yeast (dry), immobilized by polyurethane	9		37
Transformation of **5** to **6**			
bakers' yeast (dry), suspension	0		90
bakers' yeast (fresh), suspension	11		55
brewers' yeast, suspension	2		90
S. cerevisiae H-194, growing culture	48	>90	44
bakers' yeast (dry), immobilized by alginate	36	>95	46
bakers' yeast (fresh), immobilized by alginate	21	>98	53
brewers' yeast, immobilized by alginate	8		75
bakers' yeast (dry), immobilized by polyurethane	28	84	45
bakers' yeast (dry), immobilized by polyurethane containing alginate as an additive	27	80	42
baker's yeast (dry), immobilized by polyurethane containing chitosan as an additive	31	79	46

yeast simply implies a low rate of the reaction and modification of the environment might improve the situation. Thus, attempts have been made to use the bakers' yeast in immobilized form. In fact, treatment of **5** with the dry yeast entrapped in calcium alginate gel[4,5] yielded **6** in 36% yield clearly demonstrating the effect of immobilization. The *N*-ethylamide **3** was also reduced by the alginate-immobilized yeast affording a 9% yield of **4**.

The optical purities of the reduction products, **4** and **6**, were determined by ^{1}H NMR spectral analysis using chiral shift reagents. The reduction was shown to proceed with considerably high stereoselectivity (>90% e.e.) as shown in Table 1. For the purpose of determining the absolute configuration of the hydroxyl group, **4** and **6** were converted into the *O*-benzoyl derivatives. The CD spectra of the benzoates showed the same sign of Cotton effects as those of the authentic compounds having *S*-configuration which were prepared from **2**, establishing the stereochemistry of **4** and **6**.

Microbial reduction of the acetoacetamides **3** and **5** was also studied using polyurethane-immobilized yeast.[6] Paste made of dry yeast and water was admixed with a prepolymer to yield the immobilized yeast entrapped in foaming polyurethane. The polyurethane-immobilized yeast was also found to convert **3** and **5** into the hydroxy compounds **4** and **6** in 9 and 28% yields, respectively. The optical purity of the product, however, was slightly lower than that using alginate-immobilized yeast. Modification of the nature of the polyurethane foams were attempted by the addition of acidic and basic polysaccharides, alginate and chitosan, respectively. But, essentially the same results were obtained as those without polysaccharide additives.

The results are all summarized in Table 1. The starting material, **3** or **5**, was recovered in 37 to 90% yield. Use of a larger amount of yeast can be expected to improve the chemical yield of the product, but was not undertaken in the present study. During the microbial reduction glucose was added daily, which may not be necessary when the reduction proceeded slowly.

In summary, acetoacetamides were enantioselectively reduced by bakers' yeast to the corresponding hydroxy derivatives in much lower yield than acetoacetic esters. However, improvement in conversion yield was attained by immobilization of the yeast with calcium alginate gel or polyurethane foam.

Experimental

^{1}H NMR spectra were recorded on a HITACHI R-24A (60 MHz) or on a Varian XL-200 (200 MHz) spectrometer. UV and CD spectra were measured in MeOH solutions with HITACHI 124 spectrophotometer and JASCO J-40C spectropolarimeter, respectively.

Dry granular yeast ("Active Dry Yeast", S. I. LeSaffre Co.) and fresh bakers' yeast (Oriental Yeast Co.) used in the study are commercially available. *S. cerevisiae* H-194 strain was provided by Professor N. Gunge of The Kumamoto Institute of Technology.

Preparation of Immobilized Yeast. Immobilization by calcium alginate gel was done following the reported procedure.[4] Polyurethane-immobilized bakers' yeast was prepared as follows: Prepolymer (20 g) containing ca. 2 mmol g^{-1} of free NCO group prepared from polyethylene oxide (mol wt ca. 1000) and methylphenylenediisocyanate was added to an intimate mixture of dry bakers' yeast (6.5 g) and H_2O (13 cm^3) and was stirred vigorously for 20 s. After standing for 1 h the resulting polyurethane foam (ca. 200 cm^3) was cut into pieces and washed with water. For the cases with polysaccharide additives, sodium alginate (83 mg) in H_2O (2.6 cm^3) or chitosan (86 mg) in 0.2 mol dm^{-3} AcOH aq (8 cm^3) was added to the yeast–H_2O mixture before the prepolymer addition.

Yeast-Mediated Reductions of Acetoacetamides. *N*-Ethyl-3-oxobutanamide (**3**) was prepared from diketene and ethylamine according to the literature.[7] Typical procedures for the microbial reductions of **3** and **5** are described below.

Reduction of 5 with Fresh Bakers' Yeast: A suspension of fresh bakers' yeast (20 g) in H_2O (125 cm^3) containing D-glucose (15 g) was preincubated at 30 °C for 1 h. A solution of **5** (708 mg) in EtOH (5 cm^3) was added to the suspension and the mixture was shaken at 30 °C for 7 d with daily addition of D-glucose (10 g).

Reduction of 3 with Alginate-Immobilized Bakers' Yeast: Calcium alginate-immobilized bakers' yeast, prepared from dry bakers' yeast (4.2 g) and sodium alginate (4.2 g),[4] and D-glucose (9 g) in H_2O (75 cm^3) were shaken at 30 °C for 1 h. A solution of **3** (774 mg) in EtOH (4 cm^3) was added to the mixture and shaking was continued at 30 °C for 7 d with daily addition of D-glucose (9 g).

Reduction of 5 with Polyurethane-Immobilized Bakers' Yeast: The immobilized yeast prepared from dry bakers' yeast (6.5 g) and prepolymer (20 g) was immersed in the mixture of **5** (992 mg) in EtOH (25 cm^3) and D-glucose (12 g) in H_2O (225 cm^3). The mixture was shaken at 30 °C for 7 d with daily addition of D-glucose (8 g).

Reduction of 5 with *S. cerevisiae* H-194: Sterilized culture medium (pH 5.5) comprising yeast extract (1.25 g), peptone (2.5 g), D-glucose (2.5 g), and H_2O (120 cm^3) was inoculated with *S. cerevisiae* H-194 and cultured at 30 °C for 2 d. The substrate **5** (124 mg) in EtOH (1 cm^3) was added to the culture and incubated further for 7 d with daily addition of D-glucose (7.5 g).

Extraction and Identification of the Products: The incubation mixture was filtered with the aid of celite when necessary, and the filtrate was extracted with Et_2O, AcOEt, and/or $CHCl_3$. The residue was washed with the same solvent and the washings were combined with the extract. The starting material and the reduction product were isolated by SiO_2 column chromatography ($CHCl_3$–MeOH) of the extract and were characterized by ^{1}H NMR spectra without further purification. The authentic samples of the racemic forms of **4** and **6** for the NMR and TLC comparisons were prepared from 3-butanolide by treatment with $EtNH_2$[8] and from **5** by $NaBH_4$ reduction,[9] respectively.

Determination of Optical Purity and Absolute Configuration. The optical purities of **4** and **6** were determined by 200 MHz ^{1}H NMR chiral shift studies using (+)-tris[bis[perfluoro(2-propoxypropionyl)]methanate]europium(III) and tris[3-(heptafluoropropylhydroxymethylene)-*d*-camphorato]europium(III), respectively, in $CDCl_3$ solutions.

(*S*)-3-Benzoyloxy-*N*-ethylbutanamide: Ethyl (*S*)-3-hydroxybutanoate (**2**, 528 mg), prepared by the microbial reduction of **1** using fresh bakers' yeast, was benzoylated with benzoyl chloride (1 g) and pyridine (1 cm^3) at 0 °C for 1 h. Usual work-up and SiO_2 column chromatography ($CHCl_3$) afforded ethyl (*S*)-3-(benzoyloxy)butanoate (660 mg) as a colorless oil: ^{1}H NMR (CCl_4) δ=1.13 (3H, t, J=7 Hz), 1.36 (3H, d, J=6 Hz), 2.53 (1H, dd, J=15 and 6 Hz), 2.65 (1H, dd, J=15 and 6 Hz), 4.02 (2H, q, J=7 Hz), 5.36 (1H, hexad, J=6 Hz), 7.4 (3H, m), and 7.9 (2H, m). The benzoate (354 mg) in MeOH (4 cm^3) and 1 mol dm^{-3} NaOH aq (1.28 cm^3) was stirred at 0 °C for 1 h. The mixture was acidified with HCl aq, evaporated, and extracted with $CHCl_3$ to afford crude (*S*)-3-(benzoyloxy)butanoic acid (200 mg). The free acid, without purification, was dissolved in $SOCl_2$ (1 cm^3) and stirred for 1 h. After evaporation of $SOCl_2$ the resulting acid chloride was dissolved in $CHCl_3$ and a solution of $EtNH_2$ in Et_2O was added until the mixture became alkaline towards moistened litmus paper. Usual work-up and preparative TLC (SiO_2, 5% MeOH/$CHCl_3$) gave (*S*)-3-benzolyoxy-*N*-ethylbutanamide (50 mg): mp 58 °C; ^{1}H NMR (CCl_4) δ=1.03 (3H, t, *J*=7 Hz), 1.35 (3H, d, *J*=6 Hz), 2.46 (1H, dd, *J*=13 and 6 Hz), 2.62 (1H, dd, *J*=13 and 6 Hz), 3.15 (2H, quint, *J*=6 Hz), 5.40 (1H, hexad, *J*=6 Hz), 7.3 (3H, m), 7.5 (1H, br s), and 7.9 (2H, m); UV 227 nm (ε 12500); CD $[\theta]_{225}$ −4300. Anal. ($C_{13}H_{17}NO_3$) C, H, N.

(*S*)-3-Benzoyloxy-*N*-phenylbutanamide: To an ice-cooled solution of (*S*)-3-(benzoyloxy)butanoic acid (100 mg) and aniline (137 mg) in $CHCl_3$ (4 cm^3) were added dicyclohexylcarbodiimide (206 mg) and 1-hydroxybenzotriazole (32 mg) and stirred for 1 h at 0 °C and for 1 d at room temperature. Usual work-up and SiO_2 column chromatography ($CHCl_3$) afforded (*S*)-3-benzolyoxy-*N*-phenylbutanamide (65 mg) as colorless crystals: mp 116 °C; ^{1}H NMR ($CDCl_3$) δ=1.38 (3H, d, *J*=7 Hz), 2.64 (1H, dd, *J*=14 and 7 Hz), 2.78 (1H, dd, *J*=14 and 7 Hz), 5.56 (1H, hexad, *J*=7 Hz), 7.1—7.9 (10H, m), and 8.6 (1H, br s); UV 232 nm (ε 25000); CD $[\theta]_{242}$ +22000, $[\theta]_{225}$ −17000. Anal. ($C_{17}H_{17}NO_3$) C, H, N.

***O*-Benzoyl Derivatives Derived from 4 and 6:** The microbial reduction products **4** and **6** were benzoylated with benzoyl chloride–pyridine and the benzoates were isolated by SiO_2 column chromatography ($CHCl_3$). The ^{1}H NMR spectra of the benzoyl derivatives and those of the corresponding authentic samples described above were indistinguishable and their CD spectra were also very similar having the same sign of the Cotton effects.

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