

Stereochemical Control in Microbial Reduction 11. Enantioselective Reduction of β - and γ -Nitro Ketones with Bakers' Yeast

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Two useful chiral building blocks for the syntheses of natural products and other biologically important compounds, (*S*)-4-nitro-2-butanol and (*S*)-4-nitro-2-pentanol, are prepared enantioselectively by the reduction of the corresponding β - and γ -nitro ketones, respectively, with bakers' yeast.

KEY WORDS: Reduction/ Nitro alcohol/ Enantioselective/ Bakers' yeast/

INTRODUCTION

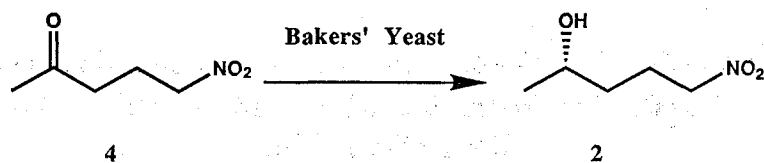
Organic nitro compounds have been used widely as important synthetic intermediates in organic syntheses because strongly electron-withdrawing property of the nitro group assists to stabilize the α -carbanion so that the carbanionic center can react smoothly with various electrophiles such as carbonyl compounds or Michael acceptors to create new carbon-carbon bonds.¹⁾ In addition, the nitro group in a newly formed composite molecule can be converted into other functional groups such as amines, alcohols, carbonyls, or hydrocarbons. Thus, optically active nitro compounds have been expected as useful chiral building blocks for the syntheses of natural products and other biologically active compounds. However, the preparation of chiral nitro compounds is neither simple nor straightforward, and reports concerning to this subject have seldom been published^{2,3)}. Therefore, a new method to synthesize a useful chiral nitro compounds are desired. We report here the enantioselective preparation of two chiral nitro alcohols, (*S*)-4-nitro-2-butanol (**7**) and (*S*)-5-nitro-2-pentanol (**2**), by the reduction of the corresponding ketones with bakers' yeast. It is worth thing that bakers' yeast is an easily obtainable and cheap reagent. Although it is a microbe, one can purchase it from bakeries or other stores, and even organic chemists who are unfamiliar to microbes can use them without difficulty.

RESULTS

Reduction of 4-Nitro-2-butanone (**3**) with Bakers' Yeast

The starting ketone, **3**, was prepared from 3-buten-2-one by treating it with so-

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Scheme 1

Table 1. Reduction of 4-Nitor-2-butanone (3) with Bakers' Yeast.^{a)}

Run	Bakers' Yeast (g)	Glucose (g)	Water (ml)	Reaction Time (d)	Yield (%)	e.e. (%)	Recovered 3 (%)
1	5	1	100	4	52	98	21
2	5	1	50	4	48	99	10
3	5	1	20	4	27	99	2
4	5	0	100	4	60	95	12
5	10	1	100	3	59	98	6
6	5	2.5	100	4	40	96	22
7	5	5	100	4	47	95	25
8	3.5 ^{b)}	1	100	6	42	98	11
9	3.5 ^{b)}	0	100	6	42	99	7

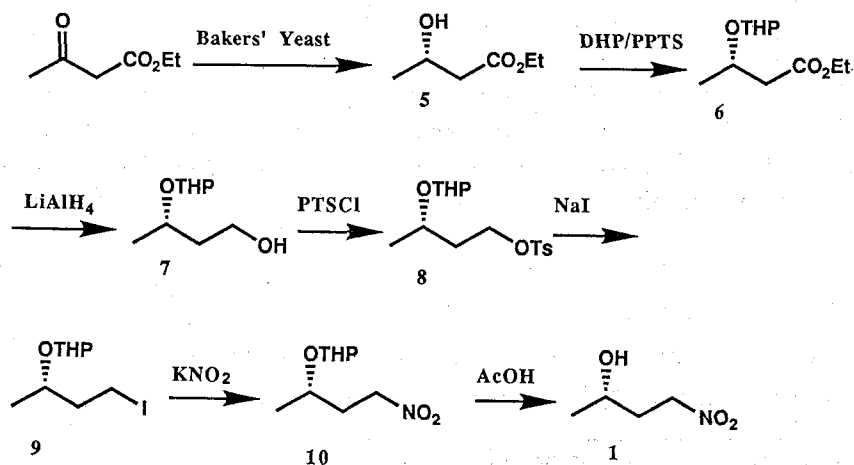
a) One mmol of **2** was used in each run.

b) Dry bal bakers' yeast was used.

dium nitrite.⁴⁾ Thus prepared **3** was reacted with bakers' yeast in water for several days. Results are listed in Table 1. Although the enantiomeric excess (e.e.) was high in every cases, the reaction conditions seem to affect the selectivity in some extent.

Determination of Absolute Configuration of **1**

To confirm the stereochemistry of **1** obtained from the yeast reduction, an authentic sample of (*S*)-**1** was prepared according to the following procedures (Scheme



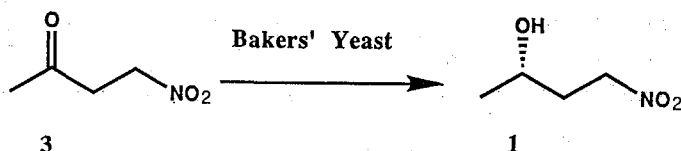
Scheme 2

2). The hydroxyl group in ethyl (*S*)-3-hydroxybutanoate (**5**), which was prepared by the yeast reduction of ethyl 3-oxobutanoate,⁵⁾ was protected by a tetrahydropyranyl group (THP) to give ethyl (*S*)-3-THPoxy-butanoate (**6**). The ester was reduced by LiAlH_4 to give (*S*)-3-THPoxybutan-1-ol (**7**), which was converted into the corresponding tosylate (**8**). The tosyl group was then substituted by an iodine to give (*S*)-3-THPoxybutyl iodide (**9**). The iodide in **9** was then substituted by a nitro group by means of the reaction with potassium nitrite to give **10**, which was subjected to acid-catalysed hydrolysis in acetic acid to yield the final product, (*S*)-**1**.

Since the signs of optical rotations of the authentic (*S*)-**1** and that of **1** obtained by the reduction of **3** with bakers' yeast were the same, the configuration of the latter was determined to be *S*.

Reduction of 5-Nitro-2-pentanone (**4**) with Bakers' Yeast

The starting ketone, **4**, was prepared by condensation of 3-buten-2-one with nitromethane in the presence of tetramethyl-guanidine in acetonitrile. The reduction of **4** with bakers' yeast proceeded more slowly than the reduction of **3**, and both chemical yield and e.c. from the former reduction were lower than those from the latter. Thus, the difference in the molecular length of only one methylene group resulted in a large difference in the reactivity and/or selectivity of biological reactions with these substrates. The results are summarized in Table 2.



Scheme 3

Table 2. Reduction of 5-Nitro-2-pentanone (**4**) with Bakers' Yeast.^{a)}

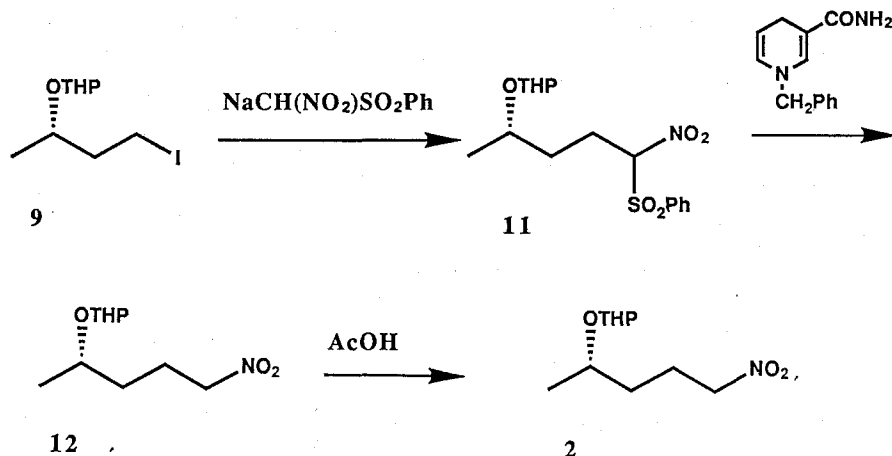
Run	Bakers' Yeast (g)	Glucose (g)	Water (ml)	Reaction Time (d)	Yield (%)	e.c. (%)	Recovered 4 (%)
1	10	1	100	7	21	90	37
2	10	1	50	7	23	94	38
3	10	1	20	7	28	95	19
4	10	5	20	7	21	96	54
5	10	0	20	7	66	94	7
6	10	0.25	20	7	40	97	7
7	5b)	1	20	7	41	95	29
8	5b)	0	20	7	54	92	16

a) One mmol of **4** was used in each run.

b) Dry bakers' yeast was used.

Determination of Absolute Configuration of **2**

To determine the absolute configuration of the product from the reduction of **4**, an authentic sample of (*S*)-**2** was prepared from **9** (Scheme 4). The reaction of



Scheme 4

9 with an anion generated from phenylsulfonyl nitromethane gave the adduct, (*S*)-4-THPOxy-1-phenylsulfonyl-1-nitropentane (**11**),⁶ which was photochemically desulfonylated by the use of BNAH to give (*S*)-4-THPOxy-1-nitropentane (**12**). Acid-catalyzed deprotection from **12** afforded (*S*)-**2**.

The comparison of the signs of optical rotations of the authentic (*S*)-**2** and the product from the reduction of **5** as well as the gas chromatographic retention times of their respective MTPA esters proved that stereochemistry of these two compounds are the same, which concludes that the configuration of the reaction product is *S*.

DISCUSSION

The reduction of **3** with bakers' yeast gives the (*S*)-nitro alcohol in each case. It is interesting to note that, the reduction of ethyl 3-oxobutanoate, **3**, the stereoselectivity increases apparently with the decrease in substrate concentration (from 95–97% e.e. at 1 g/l or less to 58% e.e. at 20 g/l),⁷ whereas the change in the concentration of substrate dose not affect the stereoselectivity significantly, within the range of concentrations so far studied. However, on the other hand, chemical yield seems to be affected by the concentration of substrate: the yield is larger at a lower concentration of the substrate in the reduction of **3**, whereas the reverse is true in the reduction of **4**.

There is another and marked difference in the reduction of nitro ketones and keto esters: glucose added to the reduction system usually stimulates the reduction of keto esters to afford higher chemical yield and more D-hydroxy esters in comparison with L-isomers at higher concentrations of glucose. This probably happens because the reduction of keto esters are taken place in a couple of pentose phosphate pathway in the microbe, which produces NADPH.⁸ As seen in Tables 1 and 2, on the other hand, the effect of the concentration of glucose is only negligible in the reduction of nitro ketones.

Dry bakers' yeast works as well and, here again, the concentrations of the sub-

strate and glucose do not affect the results on e.e. and chemical yield very much. This observation may predict that, unlike the reduction of keto esters only limited number and type of enzymes participate to the reduction of nitro ketones. Probably, the nitro ketones are not favorable substrates of the enzymes contained in bakers' yeast and responsible to the present reduction. Even the enzyme(s) which can operate on these substrates may be associated by a large Michaelis constant, K_m , toward the substrate and, consequently, the concentrations of the substrate employed in the experiments were always large enough to saturate the all enzyme(s) present in the bakers' yeast and responsible to the reduction.

In the present time, it is uncertain whether the enzymes that reduce keto esters and those that operate on nitro ketones belong to the same biological systems each other or not. In addition, it has not been clarified yet whether **3** and **4** are reduced by the same enzyme system(s) or not. In order to understand more detailed mechanism of the reduction, studies on the enzyme-level is required, which is in progress in our laboratory.

EXPERIMENTAL

Instruments

^1H NMR spectra were recorded on a Varian VXR-200 (200 MHz), and a JEOL GX-400 (400 MHz) spectrometer in CDCl_3 with Me_4Si as an internal reference. Optical rotations were measured with a Perkin-Elmer 241 polarimeter.

Materials

Organic reagents were purchased from Nakalai Tesque Co. and Aldrich Chemical Co. unless otherwise indicated. Solvents and commercially available starting materials were generally used without additional purification unless otherwise indicated. Pyridine and benzene were refluxed on calcium hydride for 1 day and distilled before the use. Pressed "raw" and dry bakers' yeasts were purchased from Oriental Yeast Co. and they were stored in a refrigerator. "Raw" yeast was used within 1 week after it had been purchased because the activity of the reduction decreased on prolonged age. Dry yeast could be stored more than 1 month without decreasing the activity.

Reduction of 4-Nitro-2-butanone (**3**) with Bakers' Yeast

To a suspension of 5 g of bakers' yeast in 20 ml of water, 1 g of glucose and 1 mmol of **3** were added and the whole suspension was stirred for 4 days at room temperature, then ethyl acetate and celite (Hiflo-super Cell) were added to the suspension and filtered. The filtrate was extracted with ethyl acetate. The organic portion was washed with water, dried on anhydrous sodium sulfate, and the solvent was evaporated under reduced pressure. The residue was subjected to a column chromatography on silica gel (eluent: dichloromethane), giving 4-nitro-2-butanol (**1**). The yield and the reaction conditions are listed in Table 1.

Preparation of Authentic (*S*)-4-Nitro-2-butanol ((*S*)-**1**) Ethyl (*S*)-3-hydroxy-

butanoate ((S)-5). A suspension composed of 100 g of bakers' yeast, 100 g of glucose, and 10.40 g (80 mmol) of ethyl 3-oxobutanoate in 300 ml of water was shaken at 30°C for 24 h. Hyflo-super-cell was added to the reaction mixture and filtered. The filtrate was extracted with ethyl acetate and the organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was distilled (78–82°C/14 mm Hg) giving 5.61 g (53.1%) of (S)-5. $[\alpha]_D^{24} = +32.4^\circ$ (c 5.15, chloroform); ref.^{5) +38.5°} (c 1.0, chloroform). ^1H NMR (CDCl_3) δ 1.14–1.40 (m, 6 H), 2.34–2.55 (m, 2 H), 2.74–3.38 (s, 1 H), and 4.10–4.38 (m, 3 H). The e.e. of the product was determined to be 82.4% by gas chromatographic analysis of the corresponding (R)-MTPA derivative.

Ethyl (S)-3-hydroxybutanoate-3-(2-tetrahydropyranyl) ether ((S)-6). A mixture of (S)-5 (5.61 g, 42.5 mmol), 2,3-dihydropyran (6.00 g, 71.4 mmol) and pyridinium *p*-toluenesulfonate (300 mg) in 80 ml of dichloromethane was stirred for 10 h. The mixture was washed with aqueous sodium carbonate (10%) and water, dried (Na_2SO_4), and concentrated. The residue was distilled giving 7.74 g (84.2%) of (S)-6. ^1H NMR (CDCl_3) δ 0.95 (d, $J=5.2$ Hz, 3 H), 1.45 (d, $J=6.8$ Hz, 3 H), 1.38–1.91 (m, 6 H), 2.25–2.82 (m, 2 H), 3.66 (s, 3 H), 3.33–4.42 (m, 3 H), and 4.61–4.77 (m, 1 H).

3-(2-Tetrahydropyranyl)-(S)-1,3-butanediol ((S)-7). Into an ethereal suspension of lithium aluminium hydride (1.5 g, 35.8 mmol) stirred and cooled with an ice bath, 6 (7.74 g, 35.8 mmol) in 20 ml of ether was added dropwise. The ice bath was removed and stirring was continued at room temperature for 4 h. Then the suspension was again cooled with an ice bath and 2 ml of water, 4 ml of 10% sodium hydroxide, and 4 ml of water was added successively. The ethereal layer was separated from the mixture and washed with water, dried over Na_2SO_4 , concentrated under reduced pressure and distilled giving (S)-7 in 76.8% yield (4.79 g). bp 125–131°C/14 mm Hg. ^1H NMR (CDCl_3) δ 1.13 (d, $J=6.2$ Hz, 3 H), 1.25 (d, $J=6.4$ Hz, 3 H), 1.30–2.00 (m, 8 H), 2.35–2.50 (m, 1 H), 3.22 (t, $J=6.4$ Hz, 1 H), 3.60–4.10 (m, 5 H), and 4.5–4.75 (m, 1 H).

3-(2-Tetrahydropyranyl)-(S)-1,3-butanediol *p*-toluenesulfonate ((S)-8). Into a dried pyridine solution of 3.2 g (18.4 mmol) of (S)-7, *p*-toluenesulfonyl chloride was added at 0°C and the mixture was stirred for 12 h. Water was added to the mixture and the organic materials were extracted with ether. The ethereal solution was washed with water, dried over Na_2SO_4 , and concentrated under reduced pressure giving crude (S)-8 5.18 g, 85.9%. ^1H NMR (CDCl_3) δ 1.07 (d, $J=6.2$ Hz, 3 H), 1.18 (d, $J=6.2$ Hz, 3 H), 1.30–2.00 (m, 8 H), 2.42 (s, 3 H), 3.30–4.30 (m, 5 H), 4.85 (m, 1 H), 7.20–7.40 (m, 2 H), and 7.60–7.85 (m, 2 H). The crude product was subjected to the following reaction without further purification.

(S)-4-Iodo-2-(2-tetrahydropyranyl)butanol ((S)-9). Into an acetone solution of (S)-8 (5.18 g, 15.8 mmol) were added 5.2 g (34.7 mmol) of sodium iodide and 3.0 g of sodium hydrogenecarbonate and the mixture was stirred for 24 h in the dark. The acetone was evaporated under reduced pressure, then water and benzene were added to the residue. The benzene layer was separated from the mixture and washed with water, 10% aqueous sodium thiosulfate, and water successively. The resulted

benzene solution was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was subjected to a chromatography on a silica gel column using dichloromethane as an eluent to afford 1.5 g (33.4%) of (*S*)-**9**. ^1H NMR (CDCl_3) δ 1.12 (d, $J=6.4$ Hz, 3 H), 1.25 (d, $J=6.2$ Hz, 3 H), 1.40–2.20 (m, 8 H), 3.15–3.35 (m, 2 H), 3.40–3.60 (m, 1 H), 3.70–4.00 (m, 2 H), and 4.65–4.75 (m, 1 H).

(*S*)-4-Nitro-2-(2-tetrahydropyranyl)butanol ((*S*)-10**).** Potassium nitrite (0.884 g, 10.4 mmol) was added into a solution of **9** (2.00 g, 7.04 mmol) in 14 ml DMF and the mixture was stirred for 6 h at room temperature. Then 35 ml of water was added to the mixture. The resulted aqueous solution was extracted with hexane (5 ml $\times$ 3). The combined hexane layer was washed with water (5 ml $\times$ 4), dried over Na_2SO_4 , and concentrated under reduced pressure. The residue was chromatographed on a silica gel column using dichloromethane as an eluent affording 0.57 g (41.0%) of (*S*)-**10**. ^1H NMR (CDCl_3) δ 1.13–1.36 (m, 3 H), 1.36–1.93 (m, 6 H), 1.98–2.36 (m, 2 H), 3.37–3.58 (m, 1 H), 3.73–4.03 (m, 2 H), and 4.77–4.33 (m, 3 H).

(*S*)-4-Nitro-2-butanol ((*S*)-1**).** A mixture of (*S*)-**10** (0.571 g), acetic acid (3.40 ml), water (1.70 ml), and THF (1.70 ml) was stirred at 80°C for 3.5 h. The resulted mixture was cooled to room temperature, then water (15 ml) was added and the mixture was extracted with ether (20 ml $\times$ 3). The combined ethereal layer was washed with aqueous sodium hydrogen carbonate and water, successively, dried over Na_2SO_4 , and concentrated under reduced pressure. The residue was chromatographed on a silica gel column using dichloromethane as an eluent affording 0.211 g (59.0% yield) of (*S*)-**1**. $[\alpha]_D^{24} = +34.5$ (c 1.96 CHCl_3). Since **1** from the yeast reduction of **3** had an optical rotational value of +40.9, the configuration of **1** from the yeast reduction was determined to be *S*.

Preparation of Authentic (*S*)-5-Nitro-2-pentanol ((*S*)-**2**).

(*S*)-1-Nitro-1-phenylsulfonyl-4-(2-tetrahydropyranyl)pentanol ((*S*)-77**).** A solution of **9** (1.5 g, 5.28 mmol) and sodium phenylsulfonyl nitromethane (7.2 g, 27.7 mmol) in 12.0 ml of hexamethylphosphoric triamide (HMPA) was stirred at room temperature under an argon atmosphere. Then ice-water (20 ml) and 2 N HCl was added to adjust the pH of the mixture to 1. Dichloromethane (30 ml) was added to the mixture and the organic layer was separated and washed with 10% aqueous sodium hydroxide. The aqueous layer was acidified to pH 1 with 2 N HCl and extracted with methylenechloride, which was washed with 10% aqueous sodium hydroxide. The combined organic layer was acidified to pH 1 and washed with water, dried over Na_2SO_4 , and concentrated under reduced pressure. The residue was chromatographed on a silica gel column using dichloromethane as an eluent affording 1.12 g (53.8%) of a mixture of diastereomers of **11**. ^1H NMR (CDCl_3) δ 1.08 (d, $J=5.3$ Hz, 3 H), 1.21 (d, $J=7.0$ Hz, 3 H), 1.30–1.90 (m, 8 H), 2.20–2.6 (m, 2 H), 3.35–3.65 (m, 1 H), 3.65–3.95 (m, 2 H), 4.50–4.60 (m, 1 H), 5.50–6.15 (m, 1 H), and 7.50–8.00 (m, 1 H).

(*S*)-1-Nitro-4-(2-tetrahydropyranyl)pentanol ((*S*)-12**).** A benzene solution (70 ml) of (*S*)-**17** (240 mg, 0.609 mmol) and 1-benzyl-1,4-dihydronicotinamide (1.5 g, 6.49 mmol) was irradiated with illumination of light from a tungsten lamp under

an argon atmosphere for 3 days. Then 30 ml of water and 50 ml of ether were added to the mixture and organic materials were extracted. The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was chromatographed on a silica gel column using dichloromethane as an eluent affording 130 mg of an 1:1 mixture of (S)-**11**, the starting material, and (S)-**12**. The mixture was used to the following reaction without further purification.

(S)-5-Nitro-2-pentanol ((S)-2). A mixture of (S)-**11** and (S)-**12** (130 mg) in tetrahydrofuran (2.0 ml) was reacted with 4.0 ml of acetic acid in 2.0 ml of water at 80°C for 1 h. Then icewater and ether were added and the organic portion was extracted with ether. The ethereal layer was washed with water, aqueous sodium carbonate, and aqueous sodium chloride successively, then dried over Na_2SO_4 and concentrated under reduced pressure. The residue was subjected to a preparative gas chromatography (PEG, 1 m, 170°C) affording 4.7 mg of (S)-**2**. $[\alpha]_D^{24} = +5.97^\circ$. Since the sign of the optical rotation of **2** from the yeast reduction was plus, the absolute configuration of **2** from the yeast reduction was determined to be S.

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