Asymmetric Reduction of Nitro Olefins by Fermenting Bakers' Yeast

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Reduction of a number of 1-nitro-1-alkenes by fermenting bakers' yeast has been found to be enantioselective, resulting in the formation of optically active 1-nitroalkanes. In most cases, optical purities of the products determined by HPLC analysis of MTPA amides were as high as 83–98% ee. The optimum rate of conversion was obtained when the reaction was carried out at pH 8 and low concentration of substrates. The absolute configuration of resulting (+)-1-nitro-2-phenylpropane was determined to be *R* by comparing the specific rotation with that of an authentic specimen after reductive hydrolysis to the corresponding aldehyde.

Bakers' yeast (*Saccharomyces cerevisiae*) has been known to reduce carbonyl compounds to optically active secondary alcohols.[1] Reduction of β-keto esters[2] and α,γ-diketones[3] to optically active β-hydroxy esters and β-hydroxy ketones provide representative examples. On the other hand, few examples are known for the reduction of carbon–carbon double bonds with one or more electron-withdrawing group, such as carbonyl,[4] perfluoroalkyl,[5] and halogen,[6] with bakers' yeast. Organic nitro compounds are important synthetic intermediates because they can be easily converted to amines, carbonyl compounds, or hydrocarbons.[7] Thus, optically active nitro compounds can be expected to be useful chiral building blocks for asymmetric synthesis. Although enzymatic methods have become increasingly important in introducing chirality into synthetic substrates, they have seldom been applied to nitro compounds[8] because of their antibiotic activities.[9] The fact that bakers' yeast has the ability to hydrogenate electron-deficient carbon–carbon double bonds encouraged us to apply this method to the asymmetric hydrogenation of nitro olefins.[10]

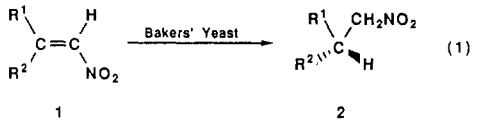
Results and Discussion

The substrates must have only hydrogen at the α-position: otherwise the carbon bearing the nitro group in the resulting saturated nitro compounds would suffer racemization under mild conditions.[8] Thus, we selected 1-nitro-2-phenylpropene (**1b**) as the first model substrate. As expected, incubation of nitroolefin **1b** with fermenting bakers' yeast afforded 1-nitro-2-phenylpropane (**2b**) in moderate yield (eq 1). The yield of **2b** was greatly influenced by the incubation time, the concentration of the substrate, and the pH of the medium. The reaction was almost complete after 48 h. The lower the concentration of the substrate, the higher the conversion of the nitro olefin **1b**. While the reaction carried out in tap water with 0.2 g/50 mL of **1b** afforded **2b** in only 44% yield, the yield of product rose to 68% when 0.05 g/50 mL of **1b** was used. In addition, use of a buffer solution as the reaction medium also improved the yield of **2b**. As is apparent from Figure 1, the best result was obtained when the reaction was carried out in a buffer at pH 8, which consisted of sodium borate–hydrochloric acid (70%, using 0.1 g of **1b** in 50 mL of the medium). The optical purity of resulting nitroalkane **2b** was determined to be 98% ee after reduction to the corresponding amine **5b**.[11]

Table I. Enantioselective Reduction of 1-Nitro-1-alkenes 1[a]

	R^1	R^2	yield,[b] %	$[\alpha]_D$,[c] deg (c, temp (°C))	ee, %
a	C_6H_5	H	26		
b	C_6H_5	Me	50	+44.3 (3.4, 27)	98[d]
c	*p*-Cl-C_6H_4	Me	48	+47.1 (2.1, 27)	89[d]
d	*p*-Br-C_6H_4	Me	57	+40.8 (1.7, 27)	94[d]
e	C_6H_5	Et	64	+38.2 (1.1, 23)	97
f	C_6H_5	*n*-Pr	23	+33.4 (1.2, 26)	89
g	C_6H_5	hexyl	0		
h	hexyl	Me (*E*)	58	+13.4 (2.2, 24)	83
i	Me	hexyl (*Z*)	48	+11.6 (2.1, 25)	66
3	$PhC(=CH_2)$-CH_2NO_2		80	+49.1 (3.4, 25)	98

[a] Incubation was carried out at room temperature for 48 h in 50 mL of tap water with 0.1 g of substrate. [b] Isolated yield. [c] Measured in $CHCl_3$. [d] See also ref 10.



(1)

1 2

	R^1	R^2
a	C_6H_5	H
b	C_6H_5	Me
c	p-Cl-C_6H_4	Me
d	p-Br-C_6H_4	Me
e	C_6H_5	Et
f	C_6H_5	n-Pr
g	C_6H_5	Hexyl
h	Hexyl	Me
i	Me	Hexyl

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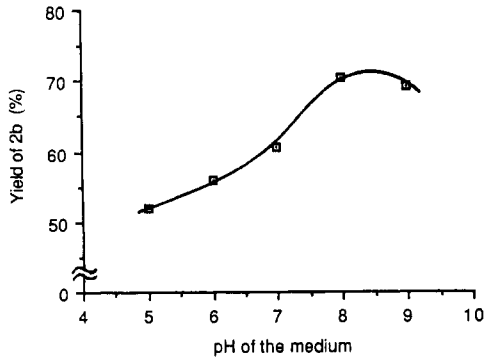


Figure 1. Effect of pH on the yield of nitroalkane **2b**. Cultivation was carried out in citric acid–Na_2HPO_4 (pH 5–7) and $Na_2B_4O_7$–HCl (pH 8–9) buffer at room temperature for 48 h. The concentration of substrate was made up to 0.1 g/50 mL.

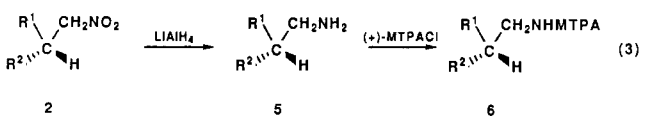
The above biological asymmetric reduction was applied to various nitro olefins (see Table I). The yields were relatively high when the R^1 was aryl or hexyl and R^2 was methyl or ethyl. In the case of 2-methyl-1-nitro-1-octene, *E* and *Z* isomers, **1h** and **1i**, could be isolated separately. The same optical isomer 2-methyl-1-nitrooctane was obtained from the *E* and *Z* isomers with fairly high enantiomeric excess (Table I, columns 8 and 9). These results are in marked contrast with the case of halo olefins reported by Utaka et al., in which the absolute configuration of the hydrogenated products depended on the *E* or *Z* stereochemistry of the starting olefins.[12] Two reasons are considered to explain the present results: isomerization of the starting olefins during reduction or enzyme selectivity for the same enantiotopic face without regard to the *E* or *Z* configuration. Although no definite information to distinguish the two mechanisms is available, the following reaction of exomethylene compound **3** is worthy of mention. The carbon–carbon double bond of 3-nitro-2-phenyl-1-propene (**3**) was also hydrogenated by incubation with bakers' yeast, although it is not conjugated with the nitro group. The absolute configuration and optical purity were shown to be the same as those of the product obtained from **1b** (Table I, column 10). The reaction path in this case is considered to involve isomerization of the double bond. The slow equilibrium between the two olefins **1b** and **3** was confirmed by a separate control experiment.

Finally, the absolute configurations of the products were determined as follows. Reduction product (+)-**2b** was converted to aldehyde (−)-**4** by reaction with sodium methoxide and titanium trichloride[13] (eq 2). Since the

(2)

2b 4

optical rotation of authentic (*S*)-**4** has been reported to be +120° (*c* 2.6, methanol),[14] it is concluded that **2b** resulting from yeast reduction has the *R* configuration. The low optical rotation of **4** is attributed to racemization of **4** during reaction. All of the other nitroalkanes exhibited positive specific rotations. To determine the optical purity, **2** was reduced to amine **5** with lithium aluminium hydride followed by conversion to amide **6** by reaction with (+)-α-methoxy-α-(trifluoromethyl)-α-phenylacetyl (MTPA)



(3)

2 5 6

chloride[15] (eq 3). The major diastereomers obtained by the above method were always the ones that have longer retention times in HPLC. These facts, together with the structural resemblance, suggest that 1-nitroalkanes **2** all have the same absolute configuration.

Experimental Section

General Procedures. ^1H NMR spectra were measured at 90 or 400 MHz. HPLC analyses were performed on Zorbax sil (4.6 mm × 250 mm) as a solid phase using UV detection at 260 nm. Capillary gas–liquid chromatography was performed on a PEG-20M bonded column (0.25 mm × 50 m). The following silica gels were used: Kieselgel 60 F_{254} (Merck) for analytical TLC, Wakogel B-5F (Wako Chemical) for preparative TLC, and silica gel 60 K070W or K230 (Katayama Chemical 230–400 mesh) for column chromatography. Melting points and boiling points are not corrected.

1-Nitro-2-phenyl-1-propene (1b) and 3-Nitro-2-phenyl-1-propene (3). To a stirred mixture of acetic anhydride (70 mL) and 60% nitric acid (10.0 g) was added 2-phenylpropene (5.01 g, 43 mmol) at 0 °C. After 20 min, the solution was poured into water (300 mL) and stirred for additional 30 min. The organic layer was washed with aqueous $NaHCO_3$ and water and dried (Na_2SO_4). Removal of the solvent under reduced pressure gave an oily residue of crude 2-acetoxy-1-nitro-2-phenylpropane,[16] which was used without purification.

A solution of the nitro acetate in triethylamine (25 mL) and chloroform (50 mL) was stirred for 3 h at room temperature. After the addition of 2 N HCl (50 mL), the mixture was extracted with dichloromethane and dried (Na_2SO_4). Removal of the solvent followed by silica gel chromatography (eluent hexane/ethyl acetate, 20/1) afforded 1-nitro-2-phenylpropene (**1b**)[17] and 3-nitro-2-phenyl-1-propene (**3**) as yellow oils.

1b: yield, 3.39 g (48%, based on 2-phenyl-1-propene); ^1H NMR (CCl_4) δ 2.60 (s, 3 H), 7.09–7.23 (m, 1 H), 7.30–7.52 (m, 5 H); IR (film) 3100, 2825, 1950, 1680, 1610, 1570, 1550, 1510, 1440, 1330, 1250, 1070, 1020, 920, 830, 760, 730, 690, 600 cm^{-1}; MS *m/e* (rel intensity) 163 (26, M^+), 133 (26), 131 (12), 117 (42), 115 (100), 105 (31), 91 (65), 77 (17).

3: yield, 1.14 g (16%, based on 2-phenyl-1-propene); ^1H NMR (CCl_4) δ 5.20 (m, 2 H), 5.42 (m, 1 H), 5.70 (m, 1 H), 7.20–7.46 (m, 5 H); IR (film) 3060, 2930, 1630, 1550, 1430, 1370, 1205, 920, 780, 740, 700 cm^{-1}; MS *m/e* (rel intensity) 163 (9, M^+), 133 (40), 117 (62), 115 (100), 91 (65), 77 (17), 51 (14).

2-Aryl-1-nitro-1-alkenes (1c–g), General Procedure. To a stirred solution of BuLi (1.56 mol/L in hexane, 13 mL) in dry diethyl ether (60 mL) was added methyltriphenylphosphonium bromide (7.14 g, 20 mmol). After 4 h, 1-aryl-1-alkanone (22 mmol) in diethyl ether (10 mL) was added, and the mixture was refluxed overnight. The mixture was filtered, and the filtrate was washed with water and dried (Na_2SO_4). Removal of the solvent followed by silica gel chromatography afforded the 2-aryl-1-alkene. 2-Aryl-1-alkenes thus obtained were converted to 2-aryl-1-nitro-1-alkenes according to the procedures described for the preparation of 1-nitro-2-phenylpropene (**1b**). In the preparation of **1e–g**, it was necessary to use aqueous $NaHCO_3$ instead of Et_3N for the elimination of acetic acid from 2-acetoxy-1-nitro-2-arylpropane.

1-Nitro-2-phenyl-1-butene (1e): yield, 0.540 g (15%, based on 1-phenyl-1-propanone); yellow oil; ^1H NMR (CCl_4) δ 1.13 (t, *J* = 7.5 Hz, 3 H), 3.03 (q, *J* = 7.5 Hz, 2 H), 7.06 (m, 1 H), 7.40 (m, 5 H); IR (film) 3100, 1610, 1570, 1330 cm^{-1}; MS *m/e* (rel intensity) 177 (28, M^+), 133 (54), 115 (53), 91 (100), 77 (31).

1-Nitro-2-phenyl-1-pentene (1f): yield, 0.510 g (31%, based on 1-phenyl-1-butanone); yellow oil; ^1H NMR (CCl_4) δ 0.97 (t, *J*

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= 7.5 Hz, 3 H), 1.30–1.70 (m, 2 H), 3.02 (t, J = 7.5 Hz, 2 H), 7.06 (m, 1 H), 7.35 (m, 5 H); IR (film) 3100, 1610, 1570, 1340 cm^{-1}; MS m/e (rel intensity) 191 (21, M^+), 147 (28), 115 (57), 91 (100), 77 (26).

1-Nitro-2-phenyl-1-octene (1g): yield, 0.550 g (24% based on 1-phenyl-1-heptanone); yellow oil; ^1H NMR (CCl_4) δ 0.85 (t, 3 H), 1.10–1.60 (m, 8 H), 3.00 (m, 2 H), 7.04 (m, 1 H), 7.35 (m, 5 H); IR (film) 3100, 1610, 1570, 1340 cm^{-1}; MS m/e (rel intensity) 233 (5, M^+), 187 (14), 133 (100), 117 (83), 91 (98), 77 (19).

(*E*)- and (*Z*)-2-Methyl-1-nitro-1-octene (1h,i). To a stirred solution of methylsulfinylmethylsodium (55 mmol) in dimethyl sulfoxide (75 mL) was added methyltriphenylphosphonium bromide (17.85 g, 50 mmol) at 0 °C, and the stirring was continued for an additional 10 min. Then, a solution of 2-octanone (6.40 g, 50 mmol) in dimethyl sulfoxide (50 mL) was added, and the solution was stirred overnight at room temperature. The reaction mixture was poured into water, and extracted with hexane. The organic layer was washed with water, dried (Na_2SO_4), and evaporated. The crude mixture was purified by column chromatography to afford 3.54 g (55%) of 2-methyl-1-octene: ^1H NMR (CCl_4) δ 0.70–1.03 (t, 3 H), 1.06–1.53 (m, 8 H), 1.57–1.76 (m, 3 H), 1.77–2.16 (m, 2 H), 4.46–4.68 (m, 2 H); IR (film) 2930, 2860, 1455, 1375, 720 cm^{-1}. 2-Methyl-1-octene (5.11 g, 40.6 mmol) was nitrated as described for **1b** to afford crude 2-acetoxy-2-methyl-1-nitrooctane, which was used for the next step without purification. The crude nitroalkane was treated with saturated aqueous $NaHCO_3$ (40 mL, room temperature, 6 h), resulting in the formation of a mixture of geometrical isomers, **1h** and **1i**. The mixture was separated by medium-pressure column chromatography on silica gel (1.2 kg/cm^2, hexane/ethyl acetate, 50/1). **1h**: yield, 0.91 g (13%); ^1H NMR (CCl_4) δ 0.89 (t, 3 H), 1.25–1.40 (m, 6 H), 1.46–1.58 (m, 2 H), 2.16–2.21 (m, 2 H), 2.24 (d, J = 1.46 Hz, 3 H), 6.95 (d, J = 1.5 Hz, 1 H); IR (film) 3120, 2940, 2860, 1640, 1555, 1518, 1460, 1375, 1342, 1190, 825, 765, 722 cm^{-1}; MS m/e (rel intensity) 172 (2, $(M + 1)^+$), 171 (2, M^+), 154 (4), 123 (6), 69 (76), 54 (88), 43 (100), 41 (91). **1i**: yield, 0.50 g (7%); ^1H NMR (CCl_4) δ 0.90 (t, 3 H), 1.26–1.45 (m, 6 H), 1.48–1.60 (m, 2 H), 1.93 (d, J = 1.47 Hz, 3 H), 2.60–2.68 (m, 2 H), 6.93 (s, 1 H); IR (film) 3120, 2940, 2860, 1760, 1635, 1555, 1520, 1460, 1380, 1340, 1200, 960, 820, 760, 725 cm^{-1}; MS m/e (rel intensity) 172 (3, $(M + 1)^+$), 171 (1, M^+), 154 (23), 123 (7), 69 (47), 55 (62), 43 (100), 41 (79).

The configuration of **1i** was determined to be *Z* by NOE: irradiation of the vinyl proton, vinyl methyl 7.4% gain, vinyl methylene 1.1% gain; irradiation of the vinyl methylene, vinyl proton −0.05% gain, vinyl methyl 7.9% gain; irradiation of the vinyl methyl, vinyl proton 17.0% gain, vinyl methylene 3.7% gain.

Reduction of Nitroalkene 1 with Bakers' Yeast. Dry yeast was purchased from Oriental Yeast Co. A mixture of dry yeast (10 g) and glucose (5 g) in tap water (50 mL) was stirred at room temperature for 10 min. A solution of nitroalkene **1** (0.1 g) in ethanol (0.2 mL) was added, and stirring was continued at the same temperature. After 48 h, the broth was extracted with ethyl acetate (300 mL). The organic layer was dried (Na_2SO_4) and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel. Elution with a mixture of hexane and ethyl acetate (20/1) afforded 1-nitroalkane **2** in yields cited in Table I. They gave a single spot on TLC (hexane/ethyl acetate, 10/1) and were identified spectroscopically.

1-Nitro-2-phenylbutane (2e): ^1H NMR (CCl_4) δ 0.80 (t, J = 7.5 Hz, 3 H), 1.70 (quint, 2 H), 3.28 (quint, 1 H), 4.40 (d, J = 7.5 Hz, 2 H), 7.00–7.40 (m, 5 H); IR (film) 1550, 1380 cm^{-1}; MS m/e (rel intensity) 179 (1, M^+), 132 (100), 91 (92), 77 (11).

1-Nitro-2-phenylpentane (2f): ^1H NMR (CCl_4) δ 0.86 (t, 3 H), 1.20 (m, 2 H), 1.60 (m, 2 H), 3.38 (quint, J = 7.5 Hz, 1 H), 4.38 (d, J = 7.5 Hz, 1 H), 6.96–7.43 (m, 5 H); IR (film) 1550, 1380 cm^{-1}; MS m/e (rel intensity) 193 (1, M^+), 132 (26), 118 (100), 104 (43), 91 (87), 77 (11).

2-Methyl-1-nitrooctane (2h): ^1H NMR (CCl_4) δ 0.76–1.03 (t, 3 H), 1.00 (d, J = 6.0 Hz, 3 H), 1.13–1.50 (m, 10 H), 2.00–2.53 (m, 1 H), 3.73–4.53 (m, 2 H); IR (film) 1550, 1380 cm^{-1}; MS m/e (rel intensity) 173 (2, M^+), 54 (76), 43 (100).

Isomerization of 1-Nitro-2-phenyl-1-propene (1b) and 3-Nitro-2-phenyl-1-propene (3). To a stirred solution of glucose (5 g) in tap water (50 mL) was added **1b** (0.1 g) at room temperature, and stirring was continued for 48 h, followed by extraction with diethyl ether. The organic layer was washed with water and brine and dried (Na_2SO_4). Removal of the solvent in vacuo gave a mixture of **1b** and **3**, whose ratio was determined to be 20:1 by ^1H NMR analysis.

When 3-nitro-2-phenyl-1-propene (**3**) was employed as the starting material and treated under the same conditions, the ratio of **3** and **1b** was 9:1.

Determination of Optical Purities of 1-Nitroalkanes (2). To a suspension of lithium aluminium hydride (0.2 g) in dry diethyl ether (5 mL) was added slowly a solution of **2** (0.9 mmol) in dry diethyl ether (2 mL) with stirring in an ice–water bath. After the mixture was stirred for 2 h at room temperature, the reaction was quenched by successive addition of water (0.2 mL), aqueous 15% NaOH (0.2 mL), and water (0.4 mL). The precipitate was filtered off, and the filtrate was evaporated. 1-Amino-2-arylalkane (**5**) was obtained in nearly quantitative yield.

Amine **5** (about 20 mg) and α-methoxy-α-(trifluoromethyl)-α-phenylacetyl chloride [prepared from (*R*)-(+)-MTPA] were allowed to react overnight in dry pyridine (0.1 mL) at room temperature. The mixture was poured into an aqueous solution of 15% $CuSO_4$ and extracted with diethyl ether, and the resulting MTPA amide **6** was purified by preparative TLC. HPLC analysis of this diastereomeric amide was carried out, with a Zorbax sil column and hexane–ethyl acetate (95:5–97:3, v/v) as eluent. The enantiomeric excess of **2** (except **2h**) was calculated from the area ratio of two peaks corresponding to the two diastereomers. The optical purity of **2h** was analyzed by capillary GLC of MTPA amide **6h**.

Determination of Absolute Configuration of 2b. To a solution of sodium methoxide (0.9 mmol) in dry methanol 1-nitro-2-phenylpropane (**2b**) (0.13 g, 0.8 mmol) was added, and the mixture was stirred for 5 min. This solution was added to a mixture of $TiCl_3$ (0.50 g, 3.2 mmol) and ammonium acetate (0.75 g, 9.7 mmol) in degassed water (5 mL) and stirred for 30 min. The reaction mixture was extracted with diethyl ether. The organic layer was washed with 5% aqueous solution of $NaHCO_3$ and dried (Na_2SO_4). Removal of the solvent under reduced pressure gave crude 2-phenylpropanal (**4**), which was purified by preparative TLC: yield, 0.07 g (63%); $[\alpha]^{25}_D$ −11° (*c* 2.02, methanol); ^1H NMR (CCl_4) δ 1.38 (d, J = 6.3 Hz, 3 H), 3.50 (q, J = 6.3 Hz, 1 H), 7.00–7.43 (m, 5 H), 9.50 (s, 1 H).