Asymmetric Reduction of 2-Aminobenzophenone Using Yeast, *Rhodosporidium toruloides*

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(±)-*N*-Isonicotinoyl-2-amino-5-chlorobenzhydrol (1) is a rice plant growth regulator which shortens the second leaf sheaths. One of the enantiomers, (*S*)-1, was obtained by microbiological asymmetric reduction of 2-amino-5-chlorobenzophenone using *Rhodosporidium toruloides* followed by isonicotinoylation. Several substituted benzhydrol derivatives were also prepared by use of the same biological method and converted to *N*-isonicotinoyl compounds. The growth-regulating activities of these compounds were evaluated.

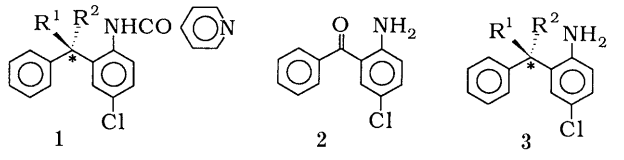
Keywords 2-aminobenzophenone; 2-aminobenzhydrol; asymmetric reduction; microbiological reduction; yeast

(±)-*N*-Isonicotinoyl-2-amino-5-chlorobenzhydrol (**1**) exhibits a strong rice plant growth-regulating activity.[1,2] For the purpose of developing a still more active compound, both of the enantiomers, (*S*)-**1** and (*R*)-**1**, have been prepared by optical resolution of (±)-2-amino-5-chlorobenzhydrol **3** using *d*-tartaric acid, followed by isonicotinoylation. Their absolute configurations have also been determined by X-ray crystallographic analysis.[3] Comparison of the growth-regulating effects of these compounds showed that (*S*)-**1** is significantly more effective, particularly at low concentrations.[2]

Thus, we wished to prepare selectively a variety of derivatives of (*S*)-**1**, as well as (*S*)-**1** itself, in order to evaluate the structure–activity relationship. However, it was considered that application of the previously used resolution method for the resolution of the (±)-**1** derivatives would be tedious. Thus, a new and efficient method for the direct synthesis of (*S*)-**1** and its derivatives was developed by use of microbial asymmetric reduction.

The asymmetric reductions of 3- or 4-nitrobenzophenone with baker's yeast have been reported[4] to produce the corresponding (*S*)-alcohols and the aminobenzophenone derivatives. Formation of the aminoalcohols has not been observed in these reductions. Thus we carried out a screening experiment[5,6] on reduction of **2** using various microorganisms such as yeasts (*Candida* species,[7] *Hansenula* species,[7] *Rhodotorula* species[7]), bacterias (*Escherichia* species, *Salmonella* species, *Bacillus* species), and molds (*Aspergillus* species, *Penicillum* species).

Reduction took place only when *Rhodosporidium toruloides*, one of the yeasts, was used in the culture medium. Even in this case, reduction proceeded very slowly and the yield was low (11% yield, 3 d at 30 °C, Table I, run 1), but the product was found to be the benzhydrol (**3**) from its infrared (IR) and nuclear magnetic resonance (NMR) spectra. The absolute structure and the optical purity of this product were determined by high-pressure liquid chromatography (HPLC) using a Chiralpac OP (Daicel) column.



(±)-**1** : racemic mixture, inabenfide
(*S*)-**1** : R¹=H, R²=OH (*S*-configuration)
(*R*)-**1** : R¹=OH, R²=H (*R*-configuration)
(*S*)-**3** : R¹=H, R²=OH
(*R*)-**3** : R¹=OH, R²=H

Chart 1

The assignment of these peaks was achieved by comparing them with those of authentic samples whose absolute configurations had been determined by X-ray crystallographic analysis (Fig. 1).[3] Namely, the peak with shorter retention time was found to correspond to that of the (*S*)-enantiomer and the peak with longer retention time to that of the (*R*)-enantiomer. Using this technique, the above reduction product was found to be the (*S*)-enantiomer and its optical purity was determined as 93%ee.

In order to establish the optimal conditions, the relation between yield and reaction period was followed.[8] As shown

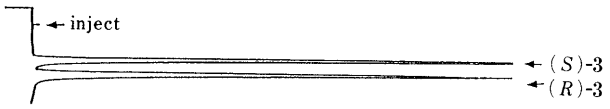


Fig. 1. HPLC Chromatogram of Each Enantiomer ((*S*)-**3** and (*R*)-**3**)

Column: Chiralpac OP (Daicel), mobile phase: 85% aqueous methanol, flow rate: 1 ml/min, detection: 254 nm.

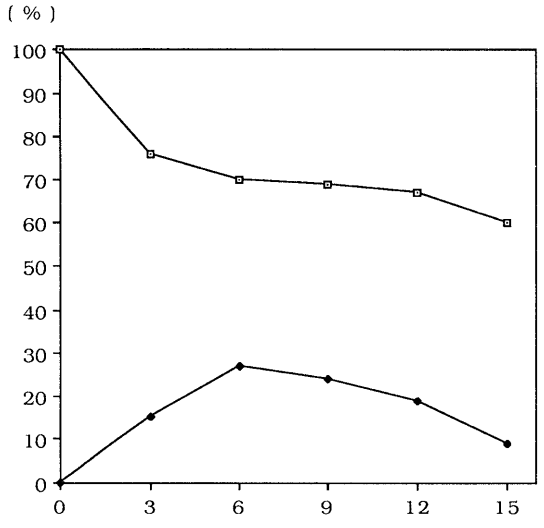


Fig. 2. Relation of Yield and Reaction Period

Substrate **2** (50 mg) in culture medium (100 ml) was incubated at 30 °C. ⊡, **2** (residual percent); ◆, (*S*)-**3** (yield percent).

in Fig. 2, the yield of (*S*)-**3** increased with the decrease of **2** and became maximum after 6 to 7 d, but did not exceed 30%. Thus, our efforts were next focussed on increasing the yield of (*S*)-**3**.

In order to circumvent the low solubility of **2** in the culture medium, the effect of dispersing agents was studied. As dispersing agents, polysorbate derivatives (Tween 20, 40, 60, 80 and 85, Span 80 and 85) were selected. A mixture of **2** and a dispersing agent was added to a culture medium and the whole was shaken for 3 d at 30 °C. The results are listed in Table I. As shown in Table I, the yields of (*S*)-**3** increased when Tween 60, 80, and 85 were added (runs 4, 5, and 6). The significant feature of the effect of dispersing agents was that (S)-**3** was produced in extremely high optical yields (runs 2, 3, 4, and 5). The prolonged reaction (7 d) using Tween 80 gave the best result (60%; 99%ee).

This method was successfully applied to the preparation of optically active benzhydrol derivatives (**5**) from substituted 2-aminobenzophenone derivatives (**4**). The absolute configuration of **5b** was determined as *S* by X-ray analysis (Fig. 3).[9] The absolute configurations of the other compounds, **5a**, **c**, **d**, have not been determined yet, but are presumed to be *S* since they were prepared in the same way as (*S*)-**5b**. These compounds were finally converted to the isonicotinamide derivatives (**6**), by reaction with isonicotinoyl chloride.[10]

Yields and optical purity of compounds **5** are listed in Table II.

The activity of compounds **6a—d** against rice plant growth was compared with those of (±)-**1**, (*S*)-**1**, and (*R*)-**1**. A solution of one of compounds **6** at a suitable concentration (0.1, 1, or 10 ppm) was added to germination test petri dishes, and 1 week after the seeding date the second leaf sheaths of rice were measured. The growth-regulating activity of these compounds was evaluated in terms of the ratio of the length of the second leaf sheaths to that of the control.

Table I. Effect of Adding Dispersing Agents

Run	Dispersing agent	(*S*)-**3** (mg) (Yield (%))	Optical purity (% ee)
1[a]	None	5.7 (11.3)	93
2[a]	Tween 20	7.8 (15.5)	99
3[a]	Tween 40	9.1 (18.0)	99
4[a]	Tween 60	11.7 (23.2)	99
5[a]	Tween 80	11.8 (23.4)	99
6[a]	Tween 85	12.1 (24.0)	96
7[a]	Span 80	3.3 (6.5)	90
8[a]	Span 85	5.3 (10.5)	87
9[b]	Tween 80	18.1 (59.8)	99

a) The substrate **2** (50 mg) in culture medium (100 ml) containing dispersing agent (1 ml) was incubated at 30 °C for 3 d. *b*) The substrate **2** (30 mg) in culture medium (200 ml) containing dispersing agent (0.3 ml) was incubated at 30 °C for 7 d.

Table II. Benzhydrol Derivatives **5** Prepared from Substituted 2-Aminobenzophenones **4**[a]

	Yield (%)	Optical purity (% ee)[c]	Recovery of **4** (%)
5a	10	65	69
5b[b]	40	99	48
5c	20	99	43
5d	23	99	68

a) The substrate **4** (500 mg) in culture medium (1000 ml) was incubated at 30 °C for 3 d. *b*) The configuration of **5b** was found to be *S*-form by X-ray crystallographic analysis. *c*) Optical purity was determined by HPLC.

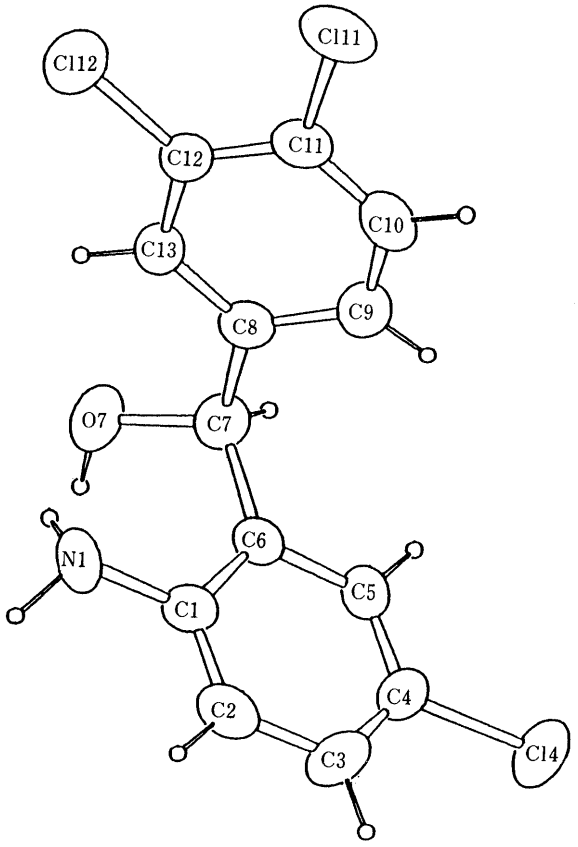
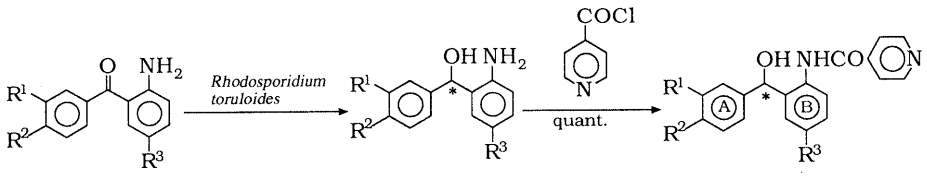


Fig. 3. ORTEP Drawing of (*S*)-**5b**



	R^1	R^2	R^3
a	H	F	Cl
b	Cl	Cl	Cl
c	H	Cl	F
d	Cl	H	Cl

Chart 2. Preparation of Substituted 2-Aminobenzhydrol and Its *N*-Isonicotinoyl Derivatives

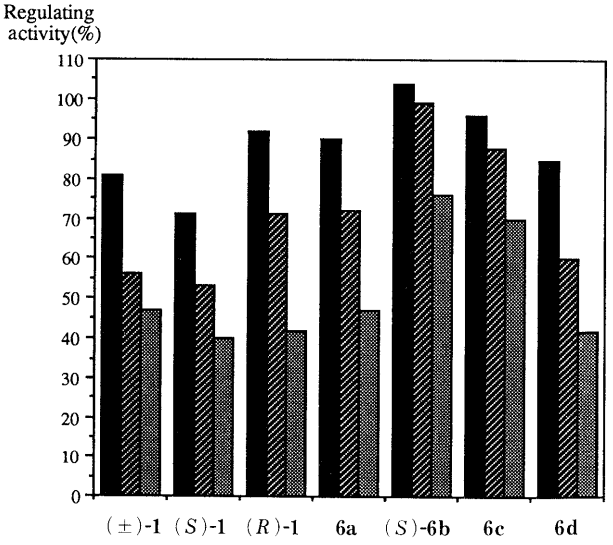


Fig. 4. Comparison of Regulating Activity of Compounds **1** and **6**
■, 0.1 ppm; ▨, 1 ppm; ▩, 10 ppm.

As reported already, the regulating activity of (±)-**1**, (*S*)-**1** and (*R*)-**1** is in the order of (*S*)-**1**>(±)-**1**>(*R*)-**1**.[2] In the cases of (*S*)-**6** having substituents on the aromatic A ring, the activities were equal to or lower than that of the (*R*)-enantiomer **1**, even though compounds **6** are in the optically active (*S*)-form which is expected to have a higher activity than the racemate or (*R*)-enantiomer.

Among the compounds so far examined, the (*S*)-isomer **1** gave the best result. Thus, a large-scale cultivation at high substrate concentration is now being examined for the large scale production of (*S*)-**1**.

Experimental

Melting points were measured with a Mettler FP61 melting point apparatus and are uncorrected. IR spectra (KBr) were measured on a Hitachi 270-30 IR spectrophotometer. NMR spectra were measured on a JEOL GX-400 instrument. Spectra were taken as 5—10% (w/v) solutions in $CDCl_3$ or CD_3OD with Me_4Si as an internal reference. HPLC was carried out on a Shimadzu SCL-6A (Daicel Chiralpac OP column (10 μm, 4.6 i.d. × 250 mm)). A Perkin-Elmer model 241 MC polarimeter was used to measure $[\alpha]_D$.

(*S*)-2-Amino-5-chlorobenzhydrol ((*S*)-3) Asymmetric reduction of **2** with *Rhodosporidium toruloides* was carried out according to the reported fermentation procedure.[5] A test tube (25 mm × 200 mm) containing 10 ml of culture medium comprising 5% glucose, 0.1% KH_2PO_4, 0.1% $(NH_4)_2SO_4$, 0.05% urea, 0.05% $MgSO_4 \cdot 7H_2O$, 0.05% $CaCl_2 \cdot 2H_2O$, 0.1% yeast extract, a trace of mineral solution (0.1% $FeSO_4 \cdot 7H_2O$, 0.1% $MnCl_2 \cdot 4H_2O$, 0.1% $ZnSO_4 \cdot 7H_2O$; 0.2 ml per 100 ml of culture medium) and tap water (pH 7.0) was inoculated with *Rhodosporidium toruloides* and the yeast was cultured at 30 °C for 2 d with continuous shaking. Then 1 ml of the above seed culture was transferred to 200 ml of the same medium. After cultivation for 3d, a mixture of 30 mg of the substrate **2** and 0.3 ml of Tween 80 was added and cultivation was continued for a further 7 d under the same conditions. *n*-Hexane and ethyl acetate were added to the reaction mixture, and the whole was filtered with the aid of Celite. The organic layer of the filtrate was washed with saturated aqueous NaCl, then dried over $MgSO_4$. Removal of the solvent gave an oily product, which was subjected to preparative thin layer chromatography (silica gel, 20 cm × 20 cm: solvent, *n*-hexane–ethyl acetate (3:1)) and finally crystallized from ethyl acetate–*n*-hexane to provide (*S*)-**3** (18.1 mg, 60%) as colorless needles, mp 131—132 °C. *Anal.* Calcd for $C_{13}H_{12}ClNO$: C, 66.81; H, 5.18; Cl, 15.17; N, 5.99. Found: C, 66.98; H, 5.19; Cl, 15.43; N, 5.95. IR ν_{max} cm^{-1}: 3232 (OH), 1606 (NH_2). NMR (CD_3OD) δ: 5.66 (1H, s, CHPh), 6.56—7.29 (8H, m, Ar-H).

2-Amino-5-chloro-4′-fluorobenzhydrol (5a) The reaction was conducted in the same way as described above. Colorless needles (from ethyl acetate–*n*-hexane), mp 125—127 °C. *Anal.* Calcd for $C_{13}H_{11}ClFNO$: C, 62.04; H, 4.41; Cl, 14.09; N, 5.57. Found: C, 61.90; H, 4.36; Cl, 14.18; N, 5.48. IR ν_{max} cm^{-1}: 3180 (OH), 1606 (NH_2). NMR ($CDCl_3$) δ: 2.55 (1H, br s, OH), 3.94 (2H, br s, NH_2), 5.77 (1H, s, CHPh), 6.59—7.36 (7H, m, Ar-H).

2-Amino-5,3′,4′-trichlorobenzhydrol (5b) Colorless needles (from ethyl acetate–*n*-hexane), mp 156—158 °C. *Anal.* Calcd for $C_{13}H_{10}Cl_3NO$: C, 51.60; H, 3.33; Cl, 35.15. N, 4.63; Found: C, 51.50; H, 3.32; Cl, 35.25; N, 4.61. IR ν_{max} cm^{-1}: 3124 (OH), 1614 (NH_2). NMR ($CDCl_3$) δ: 2.62 (1H, br s, OH), 3.97 (2H, br s NH_2), 5.73 (1H, s, CHPh), 6.60—7.51 (6H, m, Ar-H).

2-Amino-4′-chloro-5-fluorobenzhydrol (5c) Colorless needles (from ethyl acetate–*n*-hexane), mp 110—112 °C. *Anal.* Calcd for $C_{13}H_{11}ClFNO$: C, 62.04; H, 4.41; Cl, 14.09; N, 5.57. Found: C, 62.05; H, 4.38; Cl, 14.08; N, 5.50. IR ν_{max} cm^{-1}: 3150 (OH), 1630 (NH_2). NMR ($CDCl_3$) δ: 2.62 (1H, br s, OH), 3.76 (2H, br s, NH_2), 5.79 (1H, s, CHPh), 6.61—7.36 (7H, m, Ar-H).

2-Amino-5,3′-dichlorobenzhydrol (5d) Colorless needles (from ethyl acetate–*n*-hexane), mp 137—138 °C. *Anal.* Calcd for $C_{13}H_{11}Cl_2NO$: C, 58.23; H, 4.13; Cl, 26.44; N, 5.22. Found: C, 58.21; H, 4.13; Cl, 26.31; N, 5.19. IR ν_{max} cm^{-1}: 3136 (OH), 1598 (NH_2). NMR ($CDCl_3$) δ: 2.62 (1H, br s, OH), 3.95 (2H, br s, NH_2), 5.76 (1H, s, CHPh), 6.59—7.41 (7H, m, Ar-H).

(*S*)-*N*-Isonicotinoyl-2-amino-5-chlorobenzhydrol ((*S*)-1) A mixture of (*S*)-**3** (50 mg) and the hydrochloride of isonicotinoyl chloride (46 mg) in ethyl acetate (2 ml) was stirred for 16 h at room remperature. After the addition of saturated aqueous $NaHCO_3$, the reaction mixture was stirred for several minutes. The organic layer was washed with saturated aqueous NaCl, then dried over $MgSO_4$. Removal of the solvent gave **1b** (70 mg, 99%) as colorless needles (from ethyl acetate–*n*-hexane), mp 163—165 °C. *Anal.* Calcd for $C_{19}H_{15}ClN_2O_2$: C, 67.36; H, 4.46; Cl, 10.46; N, 8.27. Found: C, 67.20; H, 4.45; Cl, 10.65; N, 8.23. $[\alpha]_D^{22}$ +16.4° (*c*=1, MeOH). IR ν_{max} cm^{-1}: 3060 (OH), 1686 (NHCO). NMR (CD_3OD) δ: 5.87 (1H, s, CHPh), 7.11—8.61 (12H, m, Ar-H).

***N*-Isonicotinoyl-2-amino-5-chloro-4′-fluorobenzhydrorol (6a)** The reaction was conducted in the same way as noted above. Colorless needles (from ethyl acetate–*n*-hexane), mp 176—177 °C. *Anal.* Calcd for $C_{19}H_{14}ClFN_2O_2$: C, 63.96; H, 3.96; Cl, 9.94; N, 7.85. Found: C, 63.94; H, 3.90; Cl, 9.98; N, 7.80. $[\alpha]_D^{22}$ +8.0° (*c*=1, MeOH). IR ν_{max} cm^{-1}: 3050 (OH), 1690 (NHCO). NMR (CD_3OD) δ: 5.95 (1H, s, CHPh), 7.00—8.62 (11H, m, Ar-H).

***N*-Isonicotinoyl-2-amino-5,3′,4′-trichlorobenzhydrol (6b)** Colorless needles (from ethyl acetate–*n*-hexane), mp 176—177 °C. *Anal.* Calcd for $C_{19}H_{13}Cl_3N_2O_2$: C, 55.98; H, 3.21; Cl, 26.09; N, 6.87. Found: C, 55.95; H, 3.24; Cl, 26.07; N, 6.89. $[\alpha]_D^{22}$ −25.9° (*c*=1, MeOH). IR ν_{max} cm^{-1}: 3080 (OH), 1683 (NHCO). NMR (CD_3OD) δ: 5.81 (1H, s, CHPh), 6.98—8.64 (10H, m, Ar-H).

***N*-Isonicotinoyl-2-amino-4′chloro-5-fluorobenzhydrol (6c)** Colorless needles (from ethyl acetate–*n*-hexane), mp 152—153 °C. *Anal.* Calcd for $C_{19}H_{14}ClFN_2O_2$: C, 63.96; H, 3.96; Cl, 9.94; N, 7.85. Found: C, 63.70; H, 4.01; Cl, 9.85; N, 7.78. $[\alpha]_D^{22}$ −18.3° (*c*=1, MeOH). IR ν_{max} cm^{-1}: 3100 (OH), 1686 (NHCO). NMR (CD_3OD) δ: 5.84 (1H, s, CHPh), 7.00—8.61 (11H, s, Ar-H).

***N*-Isonicotinoyl-2-amino-5,3′-dichlorobenzhydrol (6d)** Colorless needles (from ethyl acetate–*n*-hexane), mp 167—169 °C. *Anal.* Calcd for $C_{19}H_{14}Cl_2N_2O_2$: C, 61.14; H, 3.78; Cl, 19.00; N, 7.51. Found: C, 61.16; H, 3.78; Cl, 18.92; N, 7.34. $[\alpha]_D^{22}$ +1.6° (*c*=1, MeOH). IR ν_{max} cm^{-1}: 3070 (OH), 1689 (NHCO). NMR (CD_3OD) δ: 5.83 (1H, s, CHPh), 7.00—8.62 (11H, m, Ar-H).

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References and Notes

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8) Five reactions were started at the same time and one was quenched every three days. The starting material and product were isolated.
9) *Acta Crystallogr.*, in preparation.
10) All reactions proceeded quantitatively.