Synthesis of Optically Active α-(*p*-Chlorophenyl)pyridylmethanols with Plant Cell Cultures

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We have synthesized optically active α-(*p*-chlorophenyl)pyridylmethanols by reduction or by hydrolysis with plant cell cultures.

Key words chiral α-(*p*-chlorophenyl)pyridylmethanol; *Catharanthus roseus*; *Nicotiana tabacum*; *Daucus carota*; biotransformation

α-Pyridyl alcohol derivatives are of pharmacological interest as intermediates.[1-3] Carbinoxamine,[4] a *p*-chlorophenyl-2-pyridylmethanol derivative {2-[(4-chlorophenyl)-2-pyridinylmethoxy]-*N*,*N*-dimethylethanamine}, is an antihistamic drug whose (*S*) form is active, while the (*R*) form is inactive. Another *p*-chlorophenyl-4-pyridylmethanol derivative, 4-[1-(*p*-chlorophenyl)-1-propoxymethyl]piperidine,[5] has antimalarial activity. However, chiral synthesis of *p*-chlorophenyl-2- or -4-pyridylmethanol (**2a, b**) by chemical or biological means has not yet been reported.

In a preceding paper,[6] we reported the first chiral synthesis of **2a** and **2b** by using various methods to control the stereochemical course in baker's yeast (BY) reduction, *i.e.*, immobilization of BY (in water, in organic solvent), or addition of inhibitor (ethyl chloroacetate, allyl alcohol, *etc.*). Compound (*R*)-**2a** was obtained by using calcium alginate-immobilized BY (IMBY) to reduce the corresponding ketone in water [chemical yield (C.Y.) 77%, optical yield (O.Y.) 68%]. Compound (−)-**2b** was also obtained by reduction with free BY (FBY) in the presence of ethyl chloroacetate (C.Y. 13%, O.Y. 81%) or with IMBY in hexane (C.Y. 5%, O.Y. 96%). However, the optical and chemical yields of the alcohols were unsatisfactory.

In recent years, much attention has been paid to the ability of cultured plant cells to transform enantioselectively not only secondary metabolites, but also organic foreign substrates.[7-13] In preceding publications,[14,15] we have reported the synthesis of optically active α-phenyl-4, -3- or -2-pyridylmethanol (**2c—e**) using plant cell cultures. In the case of reduction of 3- or 2-benzoylpyridine, calcium alginate-immobilized *Catharanthus* (*C.*) *roseus* cell culture (ICRC) yielded the corresponding (−)-**2d** (C.Y. 70%, O.Y. 85%) and (*R*)-**2e** (C.Y. 40%, O.Y. 92%). Furthermore, we reported a novel method[16] for producing optically active (−)-**2d** from the corresponding racemate by ICRC (C.Y. 93%, O.Y. 100%). It is of interest that plant cell cultures can discriminate the phenyl and pyridinyl groups, despite their apparent stereochemical resemblance. In this paper, we would like to report the chiral synthesis of **2a** and **2b** with plant cell cultures.

In this work, we used suspension-cultured cells which had originally been isolated from *Nicotiana* (*N.*) *tabacum* "Bright Yellow-2", *C. roseus* and *Daucus* (*D.*) *carota*. *N. tabacum* and *C. roseus* were prepared as described in our previous papers.[14,15] Cell suspension cultures were easily formed from the root and seedling of *D. carota* after a 4- to 6-week induction period when Murashige and Skoog's (MS) medium[17] was used. Subculturing was performed every 7 d by transferring 10 ml of 1-week-old culture into 80 ml of fresh MS medium containing 10 mg/l of 2,4-dichlorophenoxy acetic acid (2,4-D) as an auxin and 3% sucrose. Incubation was done on a rotary shaker (95 rpm) at 25 °C in the dark.

The biotransformation was performed with calcium alginate-immobilized cells (*C. roseus*, *N. tabacum* and *D. carota*), all of which were entrapped in calcium alginate beads as described in our previous papers.[14,15] A substrate was added to the immobilized cells in fresh MS or B5[18] medium (80 ml per flask) and the mixture was shaken on a rotary shaker (110 rpm) at 25 °C.

First, we performed enantioselective bioreduction of 2- or 4-(4-chlorobenzoyl)pyridine (**1a, b**). As shown in Table 1, immobilized *D. carota* cells (IDCC) derived from seedling enantioselectively bioreduced compound **1a** over 17 d at 25 °C to the corresponding (*R*)-**2a** in a chemical yield of 99% with a high optical purity of 92% ee (entry 4). Another *D. carota* cell line derived from root quantitatively afforded (*R*)-**2a** over 9 d with an optical purity of 81% ee (entry 3). But, in the case of the bioreduction with immobilized *N. tabacum* (INTC) or ICRC (entries 1, 2), the reaction time was longer and the chemical and optical yields were lower than those with IDCC. Therefore, we used IDCC derived from seedling to maximize the optical yield.

As shown in Fig. 1, the time courses of bioreduction of **1a** with IDCC and IMBY were examined in terms of the optical yield of **2a**. The optical yields with IMBY were very low at low conversion, but increased with increasing bioconversion, reaching a maximum of 68% ee. But, in the case of IDCC, the optical yields were very high at low conversion, and were consistent throughout [(*R*)-**2a**: 89—92% ee].

In a preceding paper,[14] we reported the bioreduction of 4-benzoylpyridine with INTC. The optical yields of (+)-**2c** were very low at low conversion, but increased with increasing bioconversion, reaching a maximum, in the same way as in BY reduction in this paper. The reasons for these differences are presently unclear, but enantioselective bioreduction system of IDCC may be different from those of INTC or IMBY. In the case of IDCC

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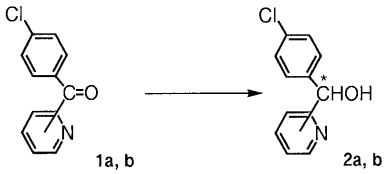
bioreduction, it is reasonable to expect, from these results, that highly stereoselective reduction of **1a** proceeds to give (*R*)-**2a** in a high optical yield.

Next, we attempted the repetitive use of IDCC. Table 1 exemplifies the bioreduction of **1a** by consecutively reusing IDCC (entries 5—7). As shown in Table 1, after four consecutive reuses, the biocatalyst still maintained the activity to generate (*R*)-**2a** [C.Y. 94%, O.Y. 86% ee]. As shown in Fig. 1, the time-courses of bioreduction by reusing IDCC were examined in terms of the chemical and optical yields. These experiments revealed that the rates of bioreduction were accelerated by consecutively reusing IDCC, while the optical yields were consistent after four consecutive reuses. It is important that we can use IDCC repeatedly in the bioreduction.

Table 1. Bioreduction of **1a** and **1b** with INTC, ICRC, IDCC, IMBY or FBY



a, 2-; b: 4-

Entry	Substrate		Time (d)	Product	% yield	% ee[a]
1	**1a**	INTC	30	**2a**	10	76 (*R*)
2	**1a**	ICRC	28	**2a**	79	10 (*R*)
3	**1a**	IDCC (root)	9	**2a**	99	81 (*R*)
4	**1a**	IDCC (seed)	17	**2a**	99	92 (*R*)
5	**1a**	IDCC second reuse	18	**2a**	99	93 (*R*)
6	**1a**	IDCC third reuse	15	**2a**	98	86 (*R*)
7	**1a**	IDCC fourth reuse	15	**2a**	94	86 (*R*)
8	**1a**	IMBY	17	**2a**	77	68 (*R*)
9	**1b**	INTC	30	**2b**	59	36 (+)
10	**1b**	ICRC	30	**2b**	50	32 (+)
11	**1b**	IDCC	20	**2b**	67	5 (+)
12	**1b**	FBY	7	**2b**	59	57 (−)

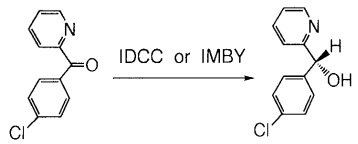
INTC, sodium alginate-immobilized *N. tabacum* cultures; ICRC, immobilized *C. roseus* cultures; IDCC, immobilized *D. carota*; FBY, free BY; IMBY, immobilized BY. *a*) Optical yields were determined by HPLC analysis. **1a** (Chiralcel OJ, 2-propanol/hexane = 1/50). **1b** (Chiralcel OB, 2-propanol/hexane = 2/3).

Next, we tried the bioreduction of **1b** with INTC, ICRC and IDCC. These results are summarized in Table 1. In the case of reduction of **1b**, optical yields of the alcohol (+)-**2b** were lower [INTC (entry 9); C.Y. 59%, O.Y. 36% ee, ICRC (entry 10); C.Y. 50%, O.Y. 32% ee, IDCC (entry 11); C.Y. 67%, O.Y. 5% ee] than that in the case of FBY (entry 12) [(−)-**2b**: C.Y. 59%, O.Y. 57% ee]. But, it is noteworthy that bioreduction of **1b** with INTC and ICRC afforded the alcohol **2b** with the opposite stereochemistry as compared with FBY.

Secondly, we tried the asymmetric hydrolysis of racemic 4-(α-acetoxy-*p*-chlorobenzyl)pyridine[6] and 2-(α-acetoxy-*p*-chlorobenzyl)pyridine[6] (**3a, b**) using Lipase AY, FBY or INTC. These results are summarized in Table 2. The hydrolysis of racemic **3a, b** with Lipase AY, FBY or INTC gave the corresponding **2a, b** (8—24% chemical and 0—65% optical yields) and recovered acetate **3a, b** (42—89% chemical and 0—12% optical yields), respectively. Optical yields of the alcohol (**2a, b**) in the case of hydrolysis were much lower than those in the case of bioreduction with IDCC.

Thus, we have succeeded in the chiral synthesis of (*R*)-**2a** with high optical purity (92% ee) by using IDCC. Furthermore, (*R*)-**2a** could be converted to (*S*)-**2a** *via* the Mitsunobu reaction.[19] When diisopropyl azodicarboxylate (DIAD) was added at 0 °C to a tetrahydrofuran (THF) solution of triphenylphosphine, acetic acid and (*R*)-**2a**, the Mitsunobu reaction occurred to give the acetate product, which was converted (*S*)-**2a** with K_2CO_3 in MeOH solution [C.Y. 86%, O.Y. 85% ee] (Chart 1).

Our findings on the chiral synthesis of **2a** and **2b** by plant cell cultures can be summarized as follows: 1) IDCC



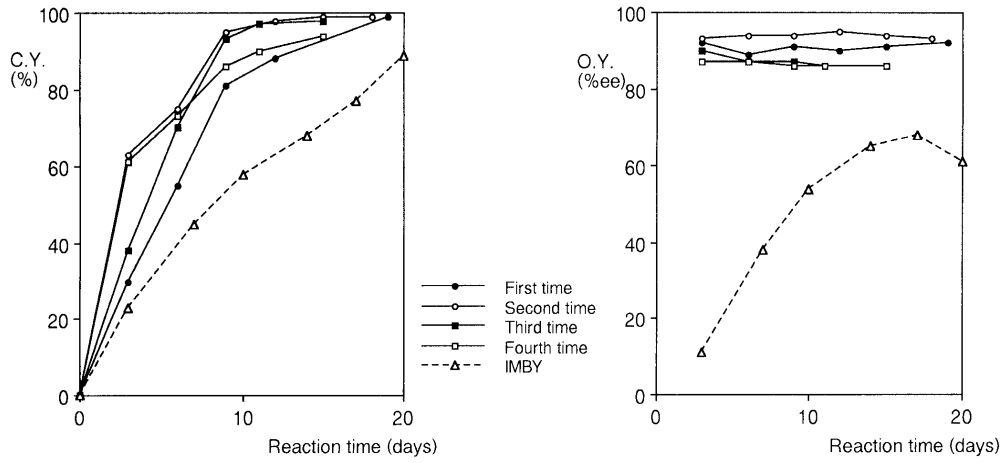
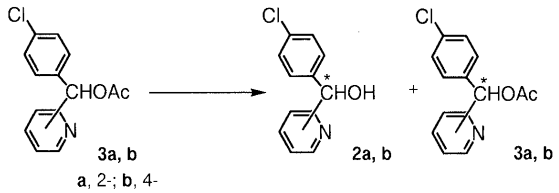


Fig. 1. Chemical and Optical Yields of (*R*)-**2a** in the Reduction by IDCC and IMBY

Table 2. Asymmetric Hydrolysis of Acetates **3a** and **3b** with INTC, Lipase AY and FBY



Entry	Substrate		Time (h)	Reacted alcohol **2a, b**		Recovered acetate **3a, b**	
				% yield	% ee	% yield	% ee[a]
1	**3a**	Lipase AY	72	24	0	68	0
2	**3a**	FBY	120	8	11 (*R*)	89	0
3	**3a**	INTC	5	21	37 (*S*)	60	12
4	**3b**	Lipase AY	72	10	65 (+)	80	7
5	**3b**	FBY	120	21	45 (−)	78	10
6	**3b**	INTC	5	21	51 (+)	70	13

a) Optical yields were determined by HPLC analysis. **3a** (Chiralcel OB, 2-propanol/hexane = 1/6). **3b** (Chiralcel OJ, 2-propanol/hexane = 1/50).

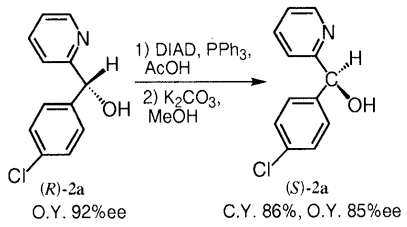


Chart 1. Conversion (*R*)-**2a** to (*S*)-**2a** by Means of the Mitsunobu Reaction

derived from seedling has high enantioselectivity in the bioreduction of **1a**. 2) The asymmetric hydrolysis with INTC is not efficient. 3) The enantioselective bioreduction system of IDCC is different from those of INTC or IMBY. 4) The repeated use of IDCC is effective for the bioreduction of **1a** from the viewpoints of optical yield and chemical yield.

Experimental

Melting points were determined on a micro-melting point apparatus (Yanagimoto) and are uncorrected. Optical rotations were measured on a JASCO DIP-140 digital polarimeter. High-performance liquid chromatography (HPLC) was carried out with a Waters 600E (ultraviolet detection) equipped with a column packed with Chiralcel OB (Daicel Chemical Industries Ltd.; 2-propanol/hexane) or Chiralcel OJ (Daicel Chemical Industries; 2-propanol/hexane). Thin layer chromatography (TLC) was performed on silica gel (Kieselgel $60F_{254}$ on aluminum sheets, Merck). All compounds were located by spraying the TLC plate with a 10% solution of phosphomolybdic acid in ethanol and heating it on a hot plate. Preparative TLC was performed on preparative layer chromatography plates (Kieselgel $60F_{254}$, 2 and 0.5 mm, Merck). Column chromatography was performed on silica gel (Kieselgel 60, 70-230 mesh, Merck).

Cultivation of *N. tabacum* "Bright Yellow-2" and *C. roseus* *N. tabacum* "Bright Yellow-2" and *C. roseus* were subcultivated according to reported procedures.[14,15]

Preparation of MS or B5 Medium We used MS medium containing Murashige and Skoog plant salt mixture (Nihon Pharmaceutical Co., Ltd.), *myo*-inositol (100 mg/l), glycine (2 mg/l), nicotinic acid (0.5 mg/l), pyridoxine·HCl (0.5 mg/l) and thiamine·HCl (0.1 mg/l). The culture medium was prepared with distilled and deionized water, adjusted to pH 5.8 with NaOH and HCl and autoclaved (1.1 kg/cm^2 at 121 °C for 20 min). Next, we used B5 medium containing Gamborg's B5 medium salt mixture (Nihon Pharmaceutical Co., Ltd.), *myo*-inositol (100 mg/l), nicotinic acid (1 mg/l), pyridoxine·HCl (1 mg/l), thiamine·HCl (10 mg/l), $CoCl_2 \cdot 6H_2O$ (0.225 mg/l) and $CuSO_4 \cdot 5H_2O$ (0.225 mg/l). The culture medium was prepared with distilled and deionized water, adjusted to pH 5.5 with NaOH and HCl, and autoclaved (1.1 kg/cm^2 at 121 °C for 20 min).

Preparation of *D. carota* Callus Cell suspension culture was established from root explants or seedlings of *D. carota*. The seedlings were rinsed with EtOH (30 sec) and NaOCl (2% aqueous solution), followed by washing with sterile distilled H_2O (× 5), and germinated on agar at 25 °C. After 7—14 d an explant (1 cm in length) was removed from the hypocotyl region and transferred into 10 ml of fresh MS medium containing 2,4-D (10 mg/l) as an auxin and 3% sucrose. Incubation was done on a rotary shaker (95 rpm) at 25 °C in the dark. The first subcultures took place at 2 to 3 weeks. To this culture, fresh MS medium (70 ml) containing 2,4-D (1 mg/l) and 3% sucrose was added. Subculturing was performed every 7 d by transferring 10 ml of 1-week-old culture into fresh MS medium (80 ml) containing 2,4-D (1 mg/l) and 3% sucrose. The cell suspension cultures from root explants were prepared according to the method used for seedling.

Preparation of Immobilized Cells (INTC, ICRC and IDCC) INTC and ICRC were prepared as described in our previous papers.[14,15] IDCC was prepared according to the following procedure. A 5% sodium alginate solution (80 ml) was added to freely suspended *D. carota* cells in the stationary phase (80 ml of MS medium, 14 d). The mixture was stirred until it became homogeneous. The sodium alginate mixture was added dropwise to a 0.6% $CaCl_2$ solution (1000 ml). The resulting IDCC beads, about 3—4 mm in diameter, were allowed to stand for 1 h and washed with H_2O. IDCC prepared from 20 ml of cells and broth, as described, was added to freshly prepared MS medium (80 ml per flask) containing 2,4-D (1 mg/l) and 3% sucrose, and the medium was shaken on a rotary shaker (110 rpm) in the dark at 25 °C for 2 d.

Biotransformation of Substrates (1a, b or 3a, b) with Immobilized Plant Cell Cultures A substrate (**1a, b** or **3a, b**, 30 mg) was added to precultured MS or B5 medium (80 ml) containing the immobilized cells, and the mixture was incubated at 25 °C on a rotary shaker (110 rpm) in the dark. In the case of INTC, we used MS medium containing 2,4-D (0.2 mg/l) and sucrose (3%). In the case of ICRC, we used B5 medium containing 2,4-D (1 mg/l) and sucrose (2%). In the case of IDCC, we used MS medium containing 2,4-D (1 mg/l) and sucrose (3%). At the conclusion of the reaction, the mixture was separated by filtration and the immobilized cells were washed with CH_2Cl_2. The filtrate was extracted with CH_2Cl_2, and the combined organic layer was washed with brine, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was subjected to column chromatography on SiO_2 with CH_2Cl_2 to give the corresponding compounds. The reaction time, the chemical yields and the optical yields are listed in Tables 1 and 2. (−)-(*R*)-**2a**: mp 90—91 °C, $[\alpha]_D^{23}$ −112.8 (c = 1.0, $CHCl_3$). OY 92% ee [lit.[20]: mp 95—97 °C, $[\alpha]_D^{17}$ −123.2 (c = 0.5, $CHCl_3$)].

Biotransformation of Substrates (1a, b or 3a, b) through the Consecutive Reuse of IDCC After each use, IDCC cells were separated from the reaction mixture by filtration or decantation, washed with MS medium,

and added to the next fresh MS medium (80 ml). After the medium had been precultured anew, the next batch of substrate was added. In this experiment, we used MS medium containing 2,4-D (1 mg/l) and sucrose (3%).

Time-Course Experiments on the Biotransformation of Substrates For the time-course experiments on the biotransformation, a part of the incubation mixture was pippeted out and then extracted with EtOAc. The conversion ratios and optical yields of each extract were measured by means of HPLC analyses.

Reaction of 1a, b, 3a, b with FBY or IMBY Substrate (1 mmol), dry BY (5 g, Oriental Yeast Co., Ltd.), water (50 ml) and glucose (1.25 g) were placed in a 100 ml flask and the mixture was stirred at room temperature with a magnetic stirrer. At the conclusion of the reaction, CH_2Cl_2 was added to the flask. The mixture was stirred for 30 min, and filtered with the aid of Celite. The filtrate was extracted with CH_2Cl_2, and the combined organic layer was washed with brine, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was subjected to column chromatography on SiO_2 with CH_2Cl_2 to give the corresponding compounds. The reaction time, the chemical yields and the optical yields are listed in Tables 1 and 2. IMBY was prepared according to the preceding paper.[21] Compound **1a** (1 mmol), IMBY (consisting of BY 5 g) and water (50 ml) were placed in a 100 ml flask and the mixture was stirred at room temperature with a magnetic stirrer. At the conclusion of the reaction, the mixture was filtered and IMBY was washed with CH_2Cl_2. The filtrate and washing were combined and treated as described for the method with BY.

Reaction of 3a and 3b with Lipase AY Substrate (45 mg), lipase AY (100 mg) and water (20 ml) containing isopropyl ether were placed in a 50 ml flask and the mixture was stirred at room temperarure with a magnetic stirrer. At the conclusion of the reaction, the mixture was filtered. The filtrate was extracted with CH_2Cl_2, and the combined organic layer was washed with brine, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was subjected to column chromatography on SiO_2 with CH_2Cl_2 to give the corresponding compounds. The reaction time, the chemical yields and the optical yields are listed in Table 2.

Conversion (*R*)-2a to (*S*)-2a *via* Mitsunobu Reaction A solution of DIAD (182 mg, 0.9 mmol) in THF (1 ml) was dropped into a THF solution of triphenylphosphine (236 mg, 0.9 mmol), acetic acid (54 mg, 0.9 mmol) and (−)-(*R*)-**2a** (100 mg, 0.45 mmol), in THF (10 ml) under Ar at 0 °C. The mixture was stirred vigorously at room temperature. After 4 h, the mixture was concentrated *in vacuo*. The residue was subjected to column chromatography on SiO_2 using EtOAc/hexane (1/4) to give the corresponding acetate. The acetate was dissolved in a mixture of MeOH (10 ml) and K_2CO_3. The mixture was stirred for 1.5 h, concentrated *in vacuo*. and extracted with CH_2Cl_2. The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. The residue was subjected to column chromatography on SiO_2 with EtOAc/hexane (1/4) to give (+)-(*S*)-**2a**. (+)-(*S*)-**2a**: mp 90—91 °C, $[\alpha]_D^{23}$ +105.4 (c=0.8, $CHCl_3$), O.Y. 86% ee.

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