Bioconversion of Optically Active Pyridyl Alcohols from the Corresponding Racemates with Plant Cell Cultures

Masumi TAKEMOTO* and Kazuo ACHIWA

School of Pharmaceutical Sciences, University of Shizuoka, 52–1 Yada, Shizuoka 422–8526, Japan.
Received September 25, 1997; accepted November 19, 1997

A novel method for producing optically active pyridyl alcohols from the corresponding racemates was developed. When racemic pyridyl alcohol is incubated with *Catharanthus roseus* cell cultures, chiral alcohol is obtained in high yield with excellent enantiomeric excess (ee) (deracemization of racemate). Furthermore, the kinetic resolution of racemic pyridyl alcohol *via* oxidation gave the chiral compound with high ee.

Key words chiral pyridyl alcohol; deracemization; kinetic resolution *via* oxidation; *Catharanthus roseus*; *Nicotiana tabacum*

α-Pyridyl alcohol derivatives are intermediates of some pharmacological interest[1-3] and (*S*)-(+)-α-phenyl-2-pyridylmethanol (**1c**) itself has analgetic and anticonvulsant activities.[4] The main methodologies to obtain chiral alcohols are the reduction of the corresponding ketones (by catalytic asymmetric hydrogenation, by treatment with a hydride reagent modified with a chiral auxiliary, or by the use of baker's yeast (BY) or other microorganisms) and the lipase-catalyzed resolution of the racemic alcohols *via* esterification or hydrolysis. The chiral synthesis of α-pyridyl alcohol derivatives has been reported. Kessar *et al.* have synthesized **1c** *via* metallation of BF_3–pyridine complex, but the optically active alcohol **1c** was not obtained from chiral boron compounds.[5] Inoue and his co-workers have synthesized (*S*)-**1c** by asymmetric reduction with a chiral polymethylene-bridged bis(NADH) model compound.[6] In preceding publications,[7-9] we have described the synthesis of optically active α-phenyl-4,3- or 2-pyridylmethanol (**1a, b, c**) by asymmetric reduction of the corresponding ketones with plant cell cultures or BY {BY: (−)-**1a** [chemical yield (C.Y.) 84%, optical yield (O.Y.) 90% enantiomeric excess (ee)], calcium alginate-immobilized cells of *Nicotiana tabacum* (INTC): (+)-**1a** (C.Y. 80%, O.Y. 72% ee), calcium alginate-immobilized *Catharanthus roseus* cell culture (ICRC): (−)-**1b** (C.Y. 70%, O.Y. 85% ee), (*R*)-**1c** (C.Y. 40%, O.Y. 92% ee)}. Furthermore, we have reported asymmetric hydrolysis of the acetate with INTC [(−)-**1a** (C.Y. 37%, O.Y. 83% ee), (−)-**1b** (C.Y. 35%, O.Y. 85% ee)].

In recent years, much attention has been paid to the preparation of optically active alcohols from racemates by selective oxidation of one of the enantiomers, and deracemization of racemates by chemical or biological methods. Fogagnolo and co-workers have reported the kinetic resolution of racemic 1-aryl and 1-heteroaryl ethanols *via* oxidation with BY[10] or microorganisms[11] to give the *R*-enantiomer. Neverthess, deracemization is the most promising method, because it permits 100% conversion of the starting racemates to the corresponding chiral compound, whereas the theoretical maximum yield is 50% in enzymatic or chemical resolution. However, relatively little work has been done on this approach. Hasegawa *et al.* reported some microorganisms that could convert racemic 1,2-diols to chiral diols.[12] Nakamura *et al.* reported that racemic 1-arylethanol was converted to the (*R*)-enantiomer by *Geotrichum candidum* IFO 5767 in high yield with excellent ee.[13] Very recently, we developed a novel method for producing optically active pyridyl alcohols **1a**—**f** from the corresponding racemates by the use of *Catharanthus roseus* cell culture.[14] In this paper, we would like to report on our use of plant cell cultures in detail.

In this work, we used suspension-cultured cells which had originally been isolated from *Nicotiana tabacum* "Bright Yellow-2" and *Catharanthus roseus*. These cell cultures were prepared as described in our previous papers.[8,9] The biotransformation was performed with calcium alginate-immobilized cells [*C. roseus* (ICRC) and *N. tabacum* (INTC)]. Freely suspended cells of *C. roseus* [20 g in Gamborg's B5 (B5) medium[15] 80 ml] or *N. tabacum* [30 g of cells in Murashige and Skoog's (MS) medium[16] 80 ml] in the stationary phase after 10 d of incubation were mixed with 5% sodium alginate solution (80 ml). ICRC prepared from 7 g of cells and 30 ml of broth was added to freshly prepared B5 medium (80 ml per flask) and the mixture was shaken on a rotary shaker (110 rpm) in the dark for 2 d at 25 °C. In the same way, INTC prepared from 10 g of cells and 30 ml of broth was added to freshly prepared MS medium (80 ml per flask). A substrate (35 mg) was added to the INTC-MS medium or ICRC-B5 medium and the mixture was shaken on a rotary shaker (110 pm) at 25 °C.

When racemic alcohols (±)-**1a**—**f** were exposed to INTC-MS medium or ICRC-B5 medium, 87—92% of the alcohols (**1a**—**f**) was recovered and only 0—13% of the alcohols was oxidized to the corresponding ketones (**2a**—**f**), as shown in Table 1. To our surprise, 93% of racemic (±)-**1b** and 100% of (±)-**1d** were converted to (−)-**1b** (100% ee) and (*R*)-(−)-**1d** (87% ee) with ICRC, respectively. Furthermore, it is noteworthy that (*R*)-**1d** with high optical purity (87% ee) was found in 100% yield in the reaction mixture when the racemate (±)-**1d** was incubated with ICRC. But, in the case of the racemic alcohols **1a**, **1c**, **1e** and **1f** with ICRC, the optical yields of recovered alcohol were lower. In a preceding publication,[9] we reported that the ability for enantioselective bioreduction with ICRC is in the order of **2c** (*ortho* substituent) > **2b** (*meta* substituent) ≫ **2a** (*para* substituent), whereas that with INTC is **2a** (*para*) > **2b** (*meta*)

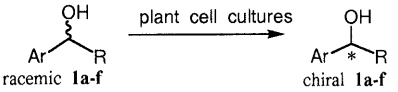
* To whom correspondence should be addressed.

>**2c** (*ortho*). But, in this biotransformation, INTC had no ability to convert a racemic alcohol to the corresponding chiral alcohol.

As shown in Fig. 1, the time courses of recovered **1b** and **1d** with ICRC were examined in terms of the chemical yield and optical yield. The enantiomeric excesses increased with incubation time, reaching maximum values of 100% ee (**1b**, 17 d) and 87% ee (**1d**, 9 d). On the other hand, the recovered yields were consistent from beginning to end [(−)-**1b**: 92—96%, (*R*)-**1d**: 100%].

When incubation were continued further, oxidation of the alcohols **1a—c** occurred to afford the corresponding ketones **2a—c**, leaving the unreacted (−)-enantiomer of the alcohols **1a—c** as shown in Table 2. In the case of **1c** with ICRC, the enantiomeric excess of **1c** increased with increasing chemical yield of **2c**, reaching a maximum of

Table 1. Optically Active Pyridyl Alcohols Production from the Corresponding Racemates by Plant Cell Culture

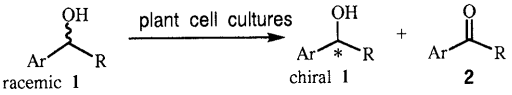


a; Ar = 4 - pyridyl; R = phenyl **d**; Ar = 4 - pyridyl; R = methyl
b; Ar = 3 - pyridyl; R = phenyl **e**; Ar = 3 - pyridyl; R = methyl
c; Ar = 2 - pyridyl; R = phenyl **f**; Ar = 2 - pyridyl; R = methyl

Substrate	Plant cell culture	Time (d)	% yield	% ee[a]	(Config.)
1a	ICRC	17	92	23	(−)
1a	INTC	17	87	15	(−)
1b	ICRC	17	**93**	**100**	(−)
1b	INTC	17	95	3	(+)
1c	ICRC	17	92	19	(*R*)
1c	INTC	17	97	5	(*R*)
1d	ICRC	9	**100**	**87**	(*R*)
1e	ICRC	12	98	11	(*S*)
1f	ICRC	10	98	0	

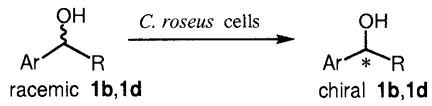
a) Optical yields were determined by HPLC analysis.

Table 2. Kinetic Resolution of the Racemic Alcohols with Plant Cell Culture *via* Oxidation



a; Ar = 4 - pyridyl; R = phenyl **c**; Ar = 2 - pyridyl; R = phenyl
b; Ar = 3 - pyridyl; R = phenyl **9**; Ar = phenyl; R = methyl

Substrate		Time (d)	**1**:**2** (ratio %)	% ee of **1**	Config.
1a	ICRC	17	92: 8	23	(−)
		38	92: 8	34	(−)
		45	82:18	38	(−)
		52	70:30	43	(−)
1a	INTC	17	85:15	10	(−)
		24	87:13	15	(−)
		32	85:15	15	(−)
		38	81:19	15	(−)
1b	ICRC	17	93: 7	100	(−)
		32	80:20	100	(−)
		38	67:33	100	(−)
		45	61:39	100	(−)
		52	55:45	100	(−)
1b	INTC	17	95: 5	3	(+)
		24	95: 5	3	(+)
		32	94: 6	3	(+)
		38	94: 6	3	(+)
		52	62:38	25	(+)
1c	ICRC	17	92: 8	19	(*R*)
		24	85:15	39	(*R*)
		32	66:34	57	(*R*)
		38	52:48	73	(*R*)
		45	43:57	85	(*R*)
		60	41:59	92	(*R*)
1c	INTC	17	97: 3	2	(*R*)
		24	92: 8	5	(*R*)
		32	93: 7	5	(*R*)
		38	92: 8	6	(*R*)
		45	88:12	6	(*R*)
		60	90:10	7	(*R*)
1g	ICRC	2	57:43	4	(*S*)
		12	25:75	37	(*S*)



b; Ar = 3 - pyridyl; R = phenyl
d; Ar = 4 - pyridyl; R = methyl

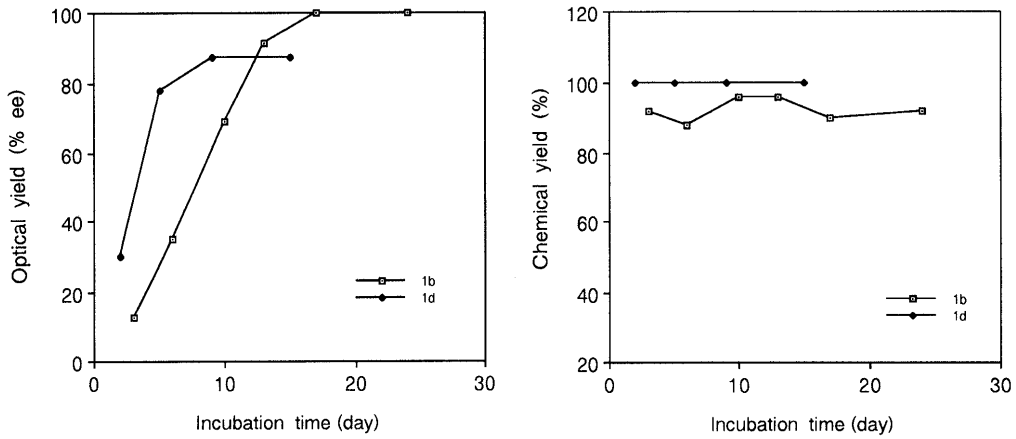


Fig. 1. Time/Courses of Optical Yields (% ee) and Chemical Yields (%) in the Biotransformation of Optically Active Pyridyl Alcohols from the Corresponding Racemates by *Catharanthus roseus* Plant Cell Culture

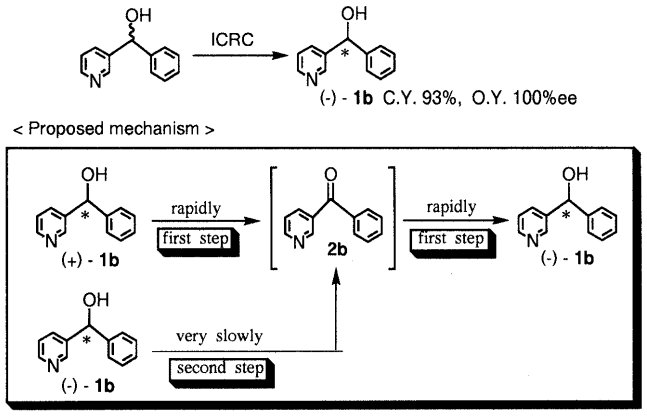


Chart 1

92% ee. This result shows the occurrence of enantioselective resolution of the racemic **1c** *via* oxidation with ICRC to give the (*R*)-(−)-enantiomer with high enantiomeric excess (92% ee). In the case of **1g**, the oxidation occurred from the beginning of the incubation to afford the ketone **2g** and (*S*)-**1g**. But, INTC had no ability for enantioselective resolution of the racemic alcohol *via* oxidation, except for (±)-**1b** (25% ee).

Although the mechanism of the deracemization and successive oxidation are not clear at present, the following mechanism seems likely in the case of **1b**, as shown in Chart 1. At first, (+)-**1b** is converted to the corresponding ketone, **2b**, which is rapidly reduced to (−)-**1b**. As shown in Fig. 1, the enantiomeric excess of **1b** increased with incubation time, but the chemical yields of **1b** were consistently 92—96% from beginning to end. These facts show that the oxidation of (+)-**1b** and the successive reduction of the ketone, **2b**, proceed relatively quickly. In contrast, (−)-**1b** is resistant to oxidation. As shown in Table 2, this oxidation requires a long time. The chemical yield of **2b** began to increase after 32 d.

Thus, we have developed two methods for the chiral synthesis of (−)-**1b**, (*R*)-**1c** and (*R*)-**1d** from the corresponding racemates with *Catharanthus roseus* cell cultures as follows.

(1) Deracemization of racemic alcohol in near-100% yield.

(2) Highly enantioselective resolution of racemic alcohol *via* oxidation. Further studies are in progress.

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 60_{F}^{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 60_{F}^{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"(NTC) *N. tabacum* "Bright Yellow-2" was subcultured every 7 d by transferring 1.3 ml of a 1-week culture into 80 ml of MS medium containing 0.2 ppm of 2,4-dichlorophenoxyacetic acid (2,4-D) and 3% sucrose (pH 5.8) on a rotary shaker (95 rpm) at 25 °C in the dark.

Cultivation of *Catharanthus roseus* Cells (CRC) Suspension cells of *Catharanthus roseus* were subcultured every 7 d by transferring a 1-week culture (8 ml) into B5 medium (80 ml) containing 2,4-D (1 ppm) and 2% sucrose (pH 5.5) on a rotary shaker (110 rpm) at 25 °C in the dark.

Preparation of ICRC and INTC A 5% sodium alginate solution (80 ml) was added to freely suspended CRC in the stationary phase (20 g of cells and B5 medium 80 ml, 10 d) or INTC (30 g of cells and MS medium 80 ml). The mixture was stirred until it became homogeneous. The resultant mixture was added dropwise to a 0.6% $CaCl_2$ solution (1000 ml). The ICRC beads, *ca.* 3—4 mm diameter, were allowed to stand for 1 h and washed with H_2O. ICRC prepared from 7 g of cell and 30 ml of broth was added to freshly prepared B5 medium (80 ml per flask) containing 2,4-D (1 ppm) and 2% sucrose, and the medium was shaken on a rotary shaker (110 rpm) in the dark at 25 °C for 2 d. In the same way, INTC prepared from 10 g of cells and 26 ml of broth was added to freshly prepared MS medium (80 ml per flask) containing 0.2 ppm of 2,4-D and 3% sucrose (pH 5.8) on a rotary shaker (110 rpm) at 25 °C in the dark.

Biotransformation of Substrates (1a—f) with ICRC or INTC A substrate (**1a—f**) (35 mg) was administered to precultured B5 medium (80 ml) containing ICRC or MS (medium) containing INTC, and the mixture was incubated at 25 °C on a rotary shaker (110 rpm) in the dark. At the conclusion of the reaction, the incubation mixture was filtered, and the ICRC or INTC beads 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. The reaction time, the chemical yield and the optical yield are listed in Tables 1 and 2. Optical yields were determined by HPLC analysis. **1a** (Chiral OB, 2-propanol/hexane = 2/3); **1b** (Chiral OB, 2-propanol/hexane = 2/3); **1c** (Chiral OJ, 2-propanol/hexane = 1/30); **1d** (Chiral OB-H, 2-propanol/hexane/diethylamine = 1/10/0.01); **1e** (Chiral OJ, 2-propanol/hexane = 1/30); **1f** (Chiral OB, 2-propanol/hexane = 1/40); **1g** (Chiral OB-H, 2-propanol/hexane = 1/9).

(−)-**1a**: mp 155—156 °C. $[\alpha]_D^{20}$ −17.5 (c = 1.20, $CHCl_3$). O.Y. 23% ee. (lit[17] mp 131—132 °C $[\alpha]_D^{18}$ −55.5 (c = 3.66, $CHCl_3$).

(−)-**1b**: mp 80—81 °C. $[\alpha]_D^{20}$ −25.0 (c = 1.30, $CHCl_3$). O.Y. 100% ee. [lit[8] mp 131—132 °C. $[\alpha]_D^{20}$ −16.9 (c = 1.38, $CHCl_3$). O.Y. 85% ee.].

(*R*)-(−)-**1c**: mp 64—65 °C. $[\alpha]_D^{23}$ −113.4 (c = 1.0, $CHCl_3$). O.Y. 92% ee. [lit.[6] (*R*)-(−)-**2c**: $[\alpha]_D^{25}$ −114.6 (c = 2.81, $CHCl_3$). O.Y. 92.7% ee.].

(*R*)-(+)-**1d**: $[\alpha]_D^{20}$ +39.2 (c = 1.10, MeOH). O.Y. 87% ee. [lit[18] $[\alpha]_D^{20}$ +42.5 (c = 1.04, MeOH) O.Y. >95% ee]

(*S*)-(−)-**1e**: $[\alpha]_D^{20}$ −8.21 (c = 1.05, $CHCl_3$). O.Y. 11% ee. [lit[18] (*R*)-(+)-**1e**: $[\alpha]_D^{20}$ +52.4 (c = 1.40, $CHCl_3$). O.Y. >95% ee; lit[19] (*S*)-(−)-**1e**: $[\alpha]_D^{20}$ −39.0 (c = 0.8, $CHCl_3$). O.Y. 67% ee].

(*S*)-(−)-**1g**: $[\alpha]_D^{20}$ −22.5 (c = 1.30, $CHCl_3$). O.Y. 37% ee. [lit[20] (*S*)-(−)-**1g**: $[\alpha]_D^{20}$ −57.1 (c = 2.74, $CHCl_3$). O.Y. 99% ee].

References

1) Yale H. Y., "Pyridine and its Derivatives," part 2, ed. by Klingsberg. E., Interscience Publishers, Inc., New York, 1961.
2) Spencer N., Papa D., Scheenk E., Sherlock M., *J. Am. Chem. Soc.*, **73**, 3856—3858 (1951).
3) Ashton M. J., Ashford A., Loveless A. H., Riddel D., Salmon J., Stevenson G. V. W., *J. Med. Chem.*, **27**, 1245—1253 (1984).
4) Frank E., Gearien J., Megahy M., Pokorny C., *J. Med. Chem.*, **14**, 551—553 (1971).
5) Kessar S. V., Singh P., Singh K. N., Dutt M., *J. Chem. Soc., Chem. Commun.*, **1991**, 570—571.
6) Seki M., Baba N., Oda J., Inoue Y., *J. Org. Chem.*, **48**, 1370—1373 (1983).
7) Takemoto M., Achiwa K., *Chem. Pharm. Bull.*, **42**, 802—805 (1994).
8) Takemoto M., Moriyasu Y., Achiwa K., *Chem. Pharm. Bull.*, **43**, 1458—1461 (1995).
9) Takemoto M., Achiwa K., Stoynov N., Chen D., Kutney J. P., *Phytochemistry*, **42**, 423—426 (1996).
10) Fantin G., Fogagnolo M., Medici A., Pedrini P., Poli S., Sinigaglia M., *Tetrahedron Lett.*, **34**, 883—884 (1993).
11) Fantin G., Fogagnolo M., Medici A., Pedrini P., Poli S., Gardini F., *Tetrahedron Asymmetry*, **4**, 1607—1612 (1993).
12) Hasegawa J., Ogura M., Tsuda S., Maemoto S., Kutsuki H., *Agric.*

Biol. Chem., **54**, 1819—1827 (1990).
13) Nakamura K., Inoue Y., Matsuda T., Ohno A., *Tetrahedron Lett.*, **36**, 6263—6266 (1995).
14) Takemoto M., Achiwa K., *Tetrahedron Asymmetry*, **6**, 2925—2928 (1995).
15) Gamborg O. L., Miller R. A., Ojima K., *Experimental Cell Research*, **50**, 151—158 (1968).
16) Murashige T., Skoog F., *Physiol. Plant*, **15**, 473—497 (1962).
17) Davies A. G., Kenyon J., Thanker K., *J. Chem. Soc.*, **1956**, 3394—3397.
18) Seemayer R., Schneider M. P., *Tetrahedron Asymmetry*, **3**, 827—830 (1992).
19) Takeshita M., Terada K., Akutsu N., *Heterocycles*, **26**, 3051—3054 (1987).
20) Naoshima Y., Akakabe Y., *Phytochemistry*, **30**, 3595—3597 (1991).