Pergamon 0031-9422(95)00915-9 *Phytochemistry*, Vol. 42, No. 2, pp. 423–426, 1996

0031-9422/96 $15.00 + 0.00

SYNTHESIS OF OPTICALLY ACTIVE α-PHENYLPYRIDYLMETHANOLS BY IMMOBILIZED CELL CULTURES OF *CATHARANTHUS ROSEUS*

MASUMI TAKEMOTO,* KAZUO ACHIWA,* NIKOLAY STOYNOV,† DAVID CHEN† and JAMES PETER KUTNEY†

School of Pharmaceutical Sciences, University of Shizuoka, 52-1 Yada, Shizuoka 422, Japan; †Department of Chemistry, The University of British Columbia, 2036 Main Mall, Vancouver, B.C., Canada V6T 1Y6

(*Received in revised form* 30 *August* 1995)

Key Word Index—*Catharanthus roseus*; Apocynaceae; *Nicotiana tabacum*; Solanaceae; biotransformation; reduction; hydrolysis; benzoylpyridine; chiral α-phenylpyridylmethanol.

Abstract—We have synthesized optically active α-phenylpyridylmethanols by reduction or hydrolysis with calcium alginate immobilized cells of *Catharanthus roseus*.

INTRODUCTION

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 [1–6]. However, there have been few examples of biotransformations of organic foreign substrates. We are interested in the feasibility of using plant cell cultures for the biotransformation of foreign substrates. In a preceding publication [7], we have reported the synthesis of optically active α-phenyl-4, 3- or 2-pyridylmethanol (**2a–c**) using *Nicotiana tabacum* cell culture (NTC) (reduction of 4-, 3- or 2-benzoylpyridine (**1a–c**) or asymmetric hydrolysis of racemic 4-, 3-, or 2-(α-acetoxybenzyl) pyridine (**3a–c**)). It was of interest to investigate whether plant cell cultures can discriminate the phenyl and pyridinyl groups of **1a–c** (reduction of carbonyl group) and **3a–c** (hydrolysis), despite their apparent stereochemical resemblance.

In the case of the reduction of **1a–c**, baker's yeast (BY) [8] and NTC yielded **2a** in a high optical purity, but **2b** and **2c** in lower optical purities. (Calcium alginate immobilized by (IMBY) in water [8]: (−)-**2a**, [chemical yield (CY). 86%, optical yield (OY). 84% ee], (−)-**2b** (CY. 65%, OY. 45% ee), (*S*)-(+)-2c (CY. 49%, OY. 28% ee). Calcium alginate immobilized NTC (INTC) in MS medium [7]: (+)-**2a** (CY. 79%, OY. 71% ee), (+)-**2b** (CY. 80%, OY. 50% ee), (*S*)-(+)-**2c** (CY. 82%, OY. 48% ee).) Furthermore, in the case of hydrolysis of racemic **3a–c**, BY yielded **2a–c** in low optical purities, however, INTC yielded **2a** and **2b** in high optical purities. [IMBY in water [8]: (−)-**2a** (CY. 30%, OY. 40% ee), (+)-**2b** (CY. 18%, OY. 33% ee), **2c** (CY. 30%, OY. 0% ee). INTC in MS medium [7]: (−)-**2a** (CY. 37%, OY. 83% ee), (−)-**2b** (CY. 35%, OY. 85% ee), **2c** (CY. 40%, OY. 0% ee).]

In general, and in accord with other studies, biocatalysts yielded high enantioselectivities with substrates that possess para substitution and low enantioselectivities when substituents were placed in the *ortho* or *meta* positions. α-Pyridyl alcohols are intermediates of pharmacological interest [9–11] and (*S*)-(+)-α-phenyl-2-pyridyl methanol [(*S*)-(+)-**2c**] itself has analgesic and anticonvulsant activities [12]. Inouye and coworkers have synthesized (*R*)-(−)-**2c** (CY. 67%, OY. 92.7% ee) by asymmetric reduction with a chiral polymethylene-bridged bis (NADH) model compound [13], but effective chiral synthesis of **2c** with a biocatalyst has not been reported. Therefore, as part of a continuing study aimed at chiral synthesis of **2b** and **2c**, we have prepared the immobilized *Catharanthus roseus* cell line (ICRC), via entrapment of cells in calcium alginate beads. There are several reports of the reduction of a C–C double bond by *C. roseus* cells [14, 15], but few reports of the reduction of carbonyl group have been presented. We are interested in the feasibility of using *C. roseus* cultures for the reduction of carbonyl groups in foreign substances and have examined the first bioreduction of the pyridyl ketones **1a–c** by ICRC.

RESULTS AND DISCUSSION

In a preceding publication [7], the bioreduction of **1a–c** was performed by three methods, that is, (A) with freely suspended NTC in the stationary phase, (B) with calcium alginate-immobilized NTC (*N. tabacum* cells only) in MS medium [16] and (C) with calcium alginate-immobilized NTC (*N. tabacum* cells and culture broth) in MS medium. The bioreduction of **1a–c** with freely suspended NTC (method A) ceased before

*Authors to whom correspondence should be addressed.

reaching complete conversion, providing the alcohols **2a–c** in a low chemical yield (3%) and in 0% optical yield. Bioreduction by method (C) proceeded at a faster rate than that by method B to yield **2a–c** in high chemical yields and high optical yields. Immobilized NTC (method C) is a favourable system for the bioreduction of **1a–c**, as determined by optical yield, chemical yield and reaction time.

In the present study, the biotransformation of **1a–c** or **3a–c** was performed with a calcium alginate-immobilized CRC system (*C. roseus* cell and culture broth). We used suspension-cultured cells that had originally been isolated from *C. roseus*. Immobilized cultured cells of *C. roseus* were prepared according to the following procedure. Freely suspended *C. roseus* (20 g of cells and B5 medium 80 ml [17]) in the stationary phase after 10 days of incubation was mixed with 5% sodium alginate solution (80 ml). The resultant mixture was dropped into a 0.6% $CaCl_2$ solution (1000 ml) and rinsed with water to give immobilized CRC (ICRC). ICRC prepared from 20 ml of cell and broth was added to freshly prepared B5 medium (80 ml per flask) and was shaken on a rotary shaker (110 rpm) in the dark for 2 days at 25°. A substrate (30 mg) was added to the INTC-B5 medium and the mixture was shaken on a rotary shaker (110 rpm) at 25°.

As shown in Table 1, ICRC enantioselectively bioreduced compounds **1c** and **1b** over 20 days at 25° to the corresponding alcohol (*R*)-(−)-**2c** and (−)-**2b**, with opposite stereochemistry to that with INTC, in high optical yield of 92% and 85% ee and chemical yield of 40% and 70%, respectively. However, ICRC reduction of **1a** was less successful and yielded racemate **2a** (5% ee) in a chemical yield of 82%. In a preceding publication [7], in the case of immobilized NTC, the optical yields of alcohol (**1a–c**) were very low at low conversion, but increased with increasing bioconversion, reaching a maximum. However, in the case of bioreduction of **1a** with ICRC, the optical yields of **1a** did not increase with increasing bioconversion.

We also tried the asymmetric hydrolysis of racemic 4-(α-acetoxybenzyl)pyridine (**3a**) [18], 3-(α-acetoxybenzyl)pyridine (**3b**) and 2-(α-acetoxybenzyl)pyridine (**3c**) [19, 20] using ICRC. As shown in Table 2, the hydrolysis of racemic **3a–c** with ICRC for 5 hr at 25° gave the corresponding (−)-**2a**, (−)-**2b** and (*R*)-(−)-**2c** with optical purities of 24% ee, 16% ee and 16% ee and chemical yields of 40%, 28% and 40%, and recovered acetate **3a**, **3b** and **3c** with optical purities of 30% ee, 7% ee and 16% ee and chemical yields of 36%, 58% and 42%, respectively.

From these data, the biotransformation of **1a–c** and **3a–c** by ICRC can be summarized as follows:

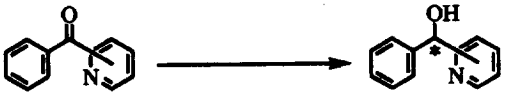
(1) The capability for enantioselective bioreduction with ICRC is **1c** (*ortho* substituent) > **1b** (*meta* substituent) ≫ **1a** (*para* substituent) compared with that of INTC or IMBY, which is the inverse **1a** (*para*) > **1b** (*meta*) > **1c** (*ortho*). Enantioselective bioreduction of **1c** and **1b** by ICRC is very effective.

(2) The bioreduction of **1b** and **1c** with ICRC yielded the alcohols **2b** and **2c** with the opposite stereochemistry compared with INTC.

(3) The capacity for asymmetric hydrolysis with ICRC is not efficient.

In general, biocatalysts distinguish between small and large groups of substrate in providing enantioselective reduction. However, in the case of benzoyl pyridine (**1a–c**), the biocatalysts studied (BY, INTC or ICRC) discriminate between the phenyl and pyridinyl

Table 1. Bioreduction of benzoylpyridine **1a–c** with ICRC, INTC and IMBY



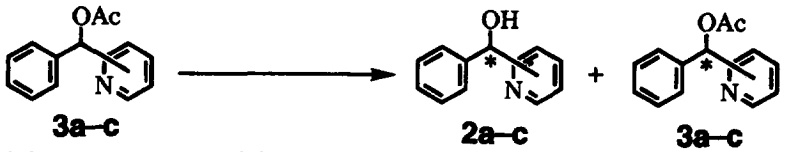
1a–c **2a–c** **(a) 4-, (b) 3-, (c) 2-**

Substrate		Product	% Yield	% ee*	Time (d)
1a	ICRC	(+)-**2a**	82	5	20
1a	INTC (C)	(+)-**2a**	79	71	15
1a	IMBY	(−)-**2a**	86	84	2
1b	ICRC	(−)-**2b**	70	85	20
1b	INTC(C)	(+)-**2b**	80	50	15
1b	IMBY	(−)-**2b**	65	45	5
1c	ICRC	(−)-**2c** (R)	40	92	20
1c	INTC (C)	(+)-**2c** (S)	82	48	12
1c	IMBY	(+)-**2c** (S)	49	28	6

ICRC: sodium alginate-immobilized *Catharanthus roseus* cultures (cell and culture broth); INTC (C): sodium alginate-immobilized *Nicotiana tabacum* (cell and culture broth); IMBY: sodium alginate immobilized baker's yeast.

*Optical yields were determined by HPLC analysis. **2a** (Chiralcel OB, 2-propanol/hexane = 2/3); **2b** (Chiralcel OB, 2-propanol/hexane = 2/3); **2c** (Chiralcel OJ, 2-propanol/hexane = 1/30).

Table 2. Asymmetric hydrolysis of acetate **3a–c** with ICRC, INTC and IMBY



(a) 4-, (b) 3-, (c) 2-

Substrate		Time (h)	Reacted alcohol (**2a–c**)		Reacted acetate (**3a–c**)	
			% Yield	% ee	% Yield	% ee*
3a	ICRC	5	40	24(−)	36	30
3a	INTC	5	37	83(−)	52	56
3a	IMBY	48	30	40(−)	51	23
3b	ICRC	5	28	16(−)	58	7
3b	INTC	5	35	85(−)	50	58
3b	IMBY	48	18	33(+)	54	10
3c	ICRC	5	40	16(−)	42	16
3c	INTC	5	40	0	42	2
3c	IMBY	48	30	0	40	2

ICRC: sodium alginate immobilized *Catharanthus roseus* cultures (cell and culture broth); INTC: immobilized *Nicotiana tabacum* cultures (cell) (B method); IMBY: immobilized baker's yeast.

*Optical yields were determined by HPLC analysis. **3a** (Chiralcel OJ, 2-propanol/hexane = 1/5). **3b** (Chiralcel OJ, 2-propanol/hexane = 2/3). **3c** (Chiralcel OJ, 2-propanol/hexane = 1/30).

group despite their apparent stereochemical resemblance. The reasons for these differences are presently unclear but may be related to the electronic environment caused by the electron-withdrawing carbonyl on the one hand versus the electron-donating basic nitrogen atom on the other. Furthermore, the enantioselectivity in the bioreduction of **1a–c** is also different between the biocatalysts INTC and ICRC. We will discuss these points in detail in a subsequent publication.

EXPERIMENTAL

General. The structures of all products were determined by interpretation of their MS and ^{1}H NMR spectra and by comparison with reported data [8]. Chemical yields refer to compounds purified by CC on silica gel. Optical rotations were measured on a JASCO DIP-140 digital polarimeter. The optical purities (% ee) of **2a–c** and **3a–c** were determined by HPLC using columns packed with Chiralcel OB (Daicel Chemical Industries Ltd, 2-PrOH–hexane) or Chiralcel OJ (Daicel Chemical Industries, 2-PrOH–hexane). Mps uncorr. All compounds were located by spraying the TLC plate with a 10% soln of phosphomolybdic acid in EtOH and heating.

Cultivation of Catharanthus roseus *cell (CRC).* Suspension cells of *C. roseus* were subcultured every 7 days by transferring 1-week-old 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° kept in the dark.

Preparation of immobilized Catharanthus roseus *cell (ICRC).* A 5% Na alginate soln (80 ml) was added to freely suspended *C. roseus* cells in the stationary phase (80 ml of B5 medium, 10 days). The mixt. was stirred until it became homogeneous. The Na alginate mixture was added dropwise to 0.6% $CaCl_2$ soln (1 l). The resulting ICRC beads, *ca* 3–4 mm diam., were allowed to stand for 1 hr and washed with H_2O. ICRC prepared from 20 ml of cells and broth, as described, 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° for 2 days.

Biotransformation of substrates (1a–c or 3a–c) with ICRC. A substrate (**1a–c** or **3a–c**) (30 mg) was administered to precultured B5 medium (80 ml) containing ICRC, and the mixt. incubated at 25° on a rotary shaker (110 rpm) in the dark. At the conclusion of the reaction, the mixture was filtered, and the ICRC beads were washed with CH_2Cl_2. The filtrate (the cultured medium from ICRC beads) was extracted with CH_2Cl_2, and the combined organic layer was washed with brine, dried over $MgSO_4$ and concd *in vacuo*. The residue was subjected to CC on SiO_2 with CH_2Cl_2 to give the corresponding phenylpyridylmethanol (**2a–c**). The reaction time, the chemical yield and the optical yield are listed in Tables 1 and 2.

(−)-**2b**. Mp 80–81°. $[\alpha]_D^{20}$ −16.9 ($CHCl_3$; *c* 1.38). O.Y. 85% ee. IR (KBr): 3346 (OH). Found: C, 77.7; H, 5.9; N, 7.6. $C_{12}H_{11}ON$ requires: C, 77.8; H, 6.0; N, 7.6%. MS *m/z*: 185 $[M]^+$. ^{1}H NMR (270 MHz, $CDCl_3$): δ 4.17 (1H, *S*, OH), 6.05 (1H, *S*, side chain H), 7.40 (1H, *dd*, $J_{4,5}$ = 7.6 Hz, $J_{5,6}$ = 3 Hz, C_5-H), 7.52 (5H, *s*, phenyl), 7.90 (1H, *tt*, $J_{4,5}$ = 7.6 Hz, $J_{2,4}$ =

2 Hz, $J_{4,6}$ = 2 Hz, C_4-H), 8.55 (1H, *dd*, $J_{4,6}$ = 2 Hz, $J_{5,6}$ = 3 Hz, C_6-H), 8.68 (1H, *d*, $J_{2,4}$ = 2 Hz, C_2-H).

(*R*)-(−)-**2c**. Mp 64–65°. $[\alpha]_D^{20}$ −113.4 ($CHCl_3$; *c* 2.50). O.Y. 92% ee {refs [13, 21] (−)-**2c**: $[\alpha]_D^{25}$ −114.6 ($CHCl_3$; *c* 2.81). O.Y. 93% ee}.

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