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Synthesis of optically active α-phenylpyridylmethanols by *Camellia sinensis* cell culture

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Abstract

(*S*)-(+)-α-phenyl-2-pyridylmethanol has analgetic and anticonvul sant activities; however, effective asymmetric synthesis by chemical or biological means has not been reported. We developed a method for producing (*S*)-(+)-α-phenyl-2-pyridylmethanol in 83% chemical yield with 86% optical yield by the repetitive use of immobilized *Camellia sinensis* cell culture. The *C. sinensis* cell culture showed similar capability for asymmetric bioreduction to that of *Catharanthus roseus* cell culture.

Keywords: *Camellia sinensis*; Cultured cells; Bioreduction; Biotransformation; Benzoylpyridine; (*S*)-(+)-α-phenyl-2-pyridylmethanol

1. Introduction

α-Pyridyl alcohol derivatives are intermediates of some pharmacological interest [1–3], and (*S*)-(+)-α-phenyl-2-pyridylmethanol (**2c**) itself has analgetic and anticonvulsant activities [4]. Kessar et al. have synthesized **2c** via metallation of a BF_3-pyridine complex, but optically active alcohol has not been obtained from chiral boron compounds. Inouye et al. have synthesized (*R*)-(−)-**2c** (92.7% e.e.) by asymmetric reduction with a chiral polymethylene-bridged bis(NADH) model compound [5]. We succeeded in asymmetric synthesis of (*R*)-(−)-**2c** (92% e.e.) by reduction of 2-benzoylpyridine (**1c**) using *Catharanthus roseus* cell culture [6].

However, chiral synthesis of (*S*)-(+)-**2c** by chemical or biological means has not yet been reported. Therefore, we continued our study aimed at chiral synthesis of (*S*)-(+)-**2c**. 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 [6–17]. Very recently, we developed a novel method for decarboxylation of *trans*-cinnamic acids by *Camellia sinensis* cell culture [17]. This led to our keen interest in the capability of *C. sinensis* cell culture for asymmetric bioreduction.

Very recently, we developed a novel synthesis of (*S*)-(+)-**2c** by the repetitive use of immobilized *C. sinensis* cell culture. In this paper, we would like to report in detail enantioselective bioreduction of 4-, 3- or 2-benzoylpyridine (**1a**–**c**) with *C. sinensis* cell culture.

2. Experimental

2.1. General experimental procedures

Melting points were determined on a micro-melting point apparatus (Yanagimoto) and are uncorrected.

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^{1}H NMR spectra were measured at 270 MHz on a JEOL JNM-EX 270 FT NMR spectrometer. Column chromatography was performed on silica gel (Kieselgel 60, 70–230 mesh, Merck).

2.2. Cultivation

Suspension cells of *C. sinensis* were cultured in 200-ml conical flasks containing 100 ml Gamborgs B5 (B5) medium [18] supplemented with 5% sucrose and 1.25 ppm 2,4-D. Cells were cultivated on a rotary shaker (110 rpm) at 25°C for 28 days prior to use for biotransformation experiments.

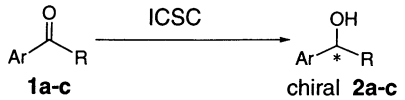
2.3. Preparation of immobilized C. sinensis cell culture (ICSC)

A 5% sodium alginate solution (80 ml) was added to freely suspended *C. sinensis* cells in the stationary phase (80 ml of B5 medium, 28 days). The mixture was stirred until it became homogenous. The sodium alginate mixture was added dropwise to 0.6% $CaCl_2$ solution (1000 ml). The resulting ICSC beads, about 3–4 mm in diameter, were allowed to stand for 1 h and washed with water. The ICSC prepared from 20 ml of cells and broth, as described, was added to freshly prepared B5 medium (80 ml per flask) containing 1.25 ppm 2,4-D and 5% sucrose, and the medium was shaken on a rotary shaker (110 rpm) in the dark at 25°C.

2.4. Asymmetric reduction of benzoylpyridines (*1a–c*) by ICSC

The total amount of 30 mg of the substrates **1a–c** was administered to the flask containing ICSC (including 8 g of CSC) in 80 ml of B5 medium. The cultures were incubated at 25°C on a rotary shaker (110 rpm). At the termination of the reaction, the immobilized cells and medium were separated by filtration. 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 alcohols **2a–c**. The reaction time, chemical yield and optical yield are listed in Table 1. Alcohols (**2a–c**) were identified by comparison of the ^{1}H NMR spectrum data with the reported data [19]. Optical purities (% e.e.) of the alcohols **2a** and **b** were determined by HPLC using columns packed with Chiralcel OB (Daicel Chemical Industries Ltd., 2-propanol/hexane = 2/3) and the alcohol **2c** was determined with Chiralcel OJ (Daicel Chemical Industries Ltd., 2-propanol/hexane = 1/30).

Table 1
Asymmetric reduction of benzoylpyridines (**la–c**) with ICSC



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

Entry	Substrate		Product	Time (days)	Yield (%)	Optical purities (% e.e.)
1	**1a**	ICSC (first)	(−)-**2a**	8	85	9
2	**1a**	ICSC (second reuse)	(−)-**2a**	4	87	12
3	**1a**	ICSC (third reuse)	(−)-**2a**	3	86	15
4	**1b**	ICSC (first)	(−)-**2b**	8	85	23
5	**1b**	ICSC (second reuse)	(−)-**2b**	4	84	65
6	**1b**	ICSC (third reuse)	(−)-**2b**	3	87	75
7	**1c**	ICSC (first)	(*S*)-(+)-**2c**	8	82	46
8	**1c**	ICSC (second reuse)	(*S*)-(+)-**2c**	4	83	86
9	**1c**	ICSC (third reuse)	(*S*)-(+)-**2c**	3	85	75

(−)-**2a**: mp 155–156°C. $[\alpha]_D^{20}$ −11.4 (c = 1.18, $CHCl_3$). O.Y. 15% e.e. lit [20] mp 131–132°C. $[\alpha]_D^{18}$ −55.5 (c = 3.66, $CHCl_3$).

(−)-**2b**: mp 80–81°C. $[\alpha]_D^{20}$ −19.0 (c = 1.20, $CHCl_3$). O.Y. 75% e.e. lit [14] mp 80–81°C. $[\alpha]_D^{20}$ −25.0 (c = 1.30, $CHCl_3$). O.Y. 100% e.e.

(*S*)-(+)-**2c**: mp 64–65°C. $[\alpha]_D^{23}$ −106.0 (c = 1.10, $CHCl_3$). O.Y. 86% e.e.

*2.5. Bioreduction of benzoylpyridines (**1a–c**) through the consecutive reuse of ICSC*

After each use, ICSC cells were separated from the reaction mixture by filtration or decantation, washed with B5 medium, and added to the next fresh B5 medium (80 ml). After the medium was precultured anew, the next batch of a substrate **1a–c** was added. In this experiment, we used B5 medium containing 1.25 ppm 2,4-D and 5% sucrose. The reaction time, chemical yield and optical yield are listed in Table 1.

3. Results and discussion

In this work, we used suspension-cultured cells which had originally been isolated from *C. sinensis*. In a preceding publication, the bioreduction of ketones**1a–c** with freely suspended *Nicotiana tabacum* cell culture ceased before reaching complete conversion, providing the alcohols in a low chemical yield [8]. Therefore, we used calcium alginate-immobilized *C. sinensis* cell culture (ICSC), which was entrapped in calcium alginate beads as described in our previous papers. A substrate was added to the immobilized cells in fresh B5 medium (80 ml per flask) and the mixture was shaken on a rotary shaker (110 rpm) at 25°C.

We performed enantioselective bioreduction of ketones **1a–c**. These results are summarized in Table 1. The ICSC enantioselectively bioreduced ketones **1a–c** over 8 days at 25°C to the corresponding alcohol (−)-**2a** (85%, 9% e.e.) (entry 1), (−)-**2b** (85%, 23% e.e.) (entry 4) and (*S*)-(+)-**2c** (82%, 46% e.e.) (entry 7), respectively. In a preceding paper, we reported the repeated use of immmobilized *Daucus carota* cells is effective for the bioreduction from the viewpoints of optical yield and chemical yield [12]. Accordingly, the repetitive use of ICSC was attempted. Table 1 exemplifies the bioreduction of **1a–c** by consecutively reusing ICSC (entries 2, 3, 5, 6, 8, 9). As expected we succeeded in shortening the reaction time with reusing ICSC. These repetitive experiments revealed that the rates of bioreduction were accelerated by consecutively reusing ICSC. Surprisingly, in the case of the ketones **1b** and **c**, optical yields of **2b** and **c** increased with the repetitive use of ICSC to reach a maximum of 75% e.e. (**2b**) (entry 6) and 86% e.e. (**2c**) (entry 8). While the reasons are presently unclear, it is reasonable to expect that the stereochemical control by the repetitive use is due to the change in the concentration and the rate of uptake of the substrate, because ICSC are surrounded tightly by calcium alginate. Thus, we have succeeded in the chiral synthesis of (*S*)-(+)-**2c** in high optical yield of 86% e.e. and chemical yield of 83%.

In general, biocatalysts distinguish between small and large groups of the substrate in providing enantioselective reduction. It was quite interesting that *C. sinensis* cell culture can discriminate between the phenyl and pyridyl groups of ketones **1b** and **c** despite their apparent stereochemical resemblance. The reasons 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 biocatalysts yielded high enantioselectivity with substrates that possess *para* substitution and low enantioselectivity when substituents were placed in the *ortho* or *meta* positions. The capability for enantioselective bioreduction with ICSC is in the order **1c** (*ortho* substituent) > **1b** (*meta* substituent) ≫ **1a** (*para* substituent) compared with that of *N. tabacum* or bakers yeast, which is the inverse **1a** (*para*) > **1b** (*meta*) > **1c** (*ortho*) [8,19]. The capability for enantioselective bioreduction with ICSC is similar to that of immobilized *C. roseus* cell culture [6]. However, the bioreduction **1a** and **c** with ICSC yielded the alcohols **2a** and **c** with the opposite stereochemistry compared with immobilized *C. roseus* cell culture [6].

We have now developed a method for producing (*S*)-(+)-**2c** in 83 with 86% optical yield by the repetitive use of ICSC.

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