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Research paper

Chemoenzymatic approaches to the synthesis of the (1*S*,2*R*)-isomer of benzyl 2-hydroxycyclohexanecarboxylate

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ABSTRACT

We examined ten strains of cultured whole-cell yeasts for the asymmetric reduction of commercially available ethyl 2-oxocyclohexanecarboxylate, and found that the (1*S*,2*S*)-stereoisomer of ethyl 2-hydroxycyclohexanecarboxylate was the major stereoisomer produced by *Williopsis californica* JCM 3600. The ethyl group of the ester was then substituted with a benzyl group with low volatility and increased hydrophobicity to facilitate the isolation of the expected product. Incubation with *W. californica* furnished benzyl (1*S*,2*S*)-2-hydroxycyclohexanecarboxylate (>99.9% ee) in 51.0% yield together with its (1*R*,2*S*)-isomer (>99.9% ee) in 35.4% yield. Upon treatment of the same substrate bearing the benzyl ester with a screening kit of purified overexpressed carbonyl reductases (Daicel Chiralscreen® OH), two enzymes (E031, E078) furnished the (1*R*,2*S*)-isomer as the major product. With another enzyme (E007), the (1*S*,2*R*)-isomer was obtained, but its ee was very low (25.6%). The highly enantiomerically enriched (1*S*,2*S*)-isomer obtained by *W. californica* was transformed to the (1*S*,2*R*)-isomer (>99.9% ee), whose availability until now has been low, in 43.3% yield over two steps involving tosylation and subsequent inversive attack with tetrabutylammonium nitrite.

1. Introduction

The stereochemically enriched form of ethyl 2-hydroxycyclohexanecarboxylate (**1a**, Fig. 1) is a versatile synthetic starting material [1–5], taking advantage of two independent functional groups neighboring on the six-membered ring. By Fráter's stereoselective alkylation [6], which can introduce an all-carbon quaternary chiral center at the C-1 position, the resulting products (Fig. 1) have widened the usefulness of this starting material [7].

To generate stereochemically enriched forms, classical enantiomeric resolutions of the racemic mixture have been developed, such as preferential crystallization of the diasteromeric salt of the corresponding racemic hydroxy acid [5]. Also, much effort has been devoted to the development of enzyme-catalyzed kinetic resolution [3,8,9].

In addition, asymmetric reduction of the corresponding β-oxoester **2a** has been examined. The reduction is generally accompanied by the racemization of the substrate, as the acidity of the proton at C-1 is high [10–12]. Ruthenium-catalyzed asymmetric hydrogenation of **2a** produces **1a** with a varied ratio between *syn*- and *anti*-diastereomers with up to 90% ee in *syn*-**1a** [13,14] and with 91% ee in *anti*-**1a** [15].

Biocatalytic asymmetric reduction of carbonyl compounds is a potent way towards the production of enantiomerically enriched compounds [16]. As shown in recent examples, whole-cell microorganisms have the reservoir of various oxidoreductive enzymes with proper co-factor regenerating systems, and can be applied to wide range of substrates [17]. Once the desirable results in regard to the stereochemistry of the products and the catalytic activity are obtained, gene and protein engineering approaches enable large-scale production of the enzymes for the specific purposes [18,19].

In this context, the reduction of **2a** has a long history. Incubation with whole-cell *Saccharomyces cerevisiae* (bakers' yeast) [20,21] furnishes (1*R*,2*S*)-**1a** as the major isomer with 86% ee in 65% isolated yield [6]. Buison and Azerad examined many kinds of incubated whole-cell microorganisms to reduce **2a**, and found that *Mucor racemosus*, *M. circinelloides*, and *Colletotrichum gloeosporoides* exhibit high diastereoselectivity [22]. The former two furnish *syn*- (1*R*,2*S*)-**1a** with 96–97% ee, while the latter strain furnishes *anti*- (1*S*,2*S*)-**1a** with >99.9% ee. However, (1*S*,2*R*)-**1a** with high ee has so far been unattainable. Later, an isolated *S. cerevisiae* carbonyl reductase was overexpressed in *Escherichia coli*, and the ee of (1*S*,2*R*)-**1a** was 80%, although the ratio of the *syn*-isomer was

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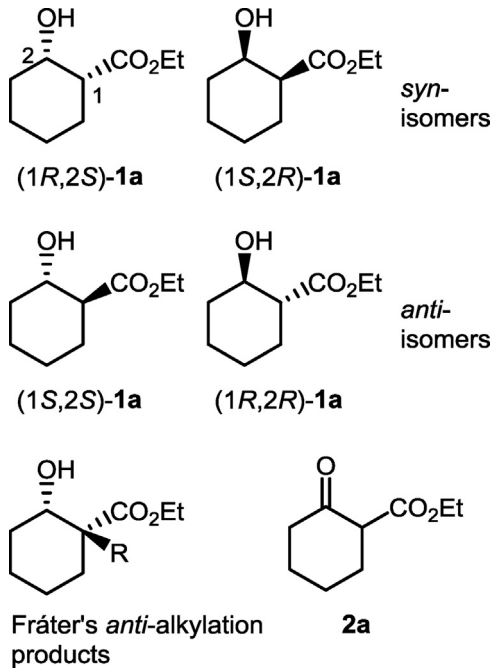

Fig. 1. Stereoisomers of ethyl 2-hydroxycyclohexanecarboxylate **1a**, the related alkylation product, and the corresponding β-oxoester **2a**, the reduction precursor.

quite high [23]. It has also been reported that the reduction of **2a** with an enzyme from a recombinant ketoreductase library, KRED-129, furnishes (1*S*,2*R*)-**1a** at 90% ee, but only at an analytical scale [24].

In this study, we performed biocatalytic asymmetric reduction by incubation with several whole-cell yeast strains [25–28] and with a commercially available carbonyl reductase screening kit, Daicel Chiralscreen® OH [29,30], to evaluate selectivity in the reduction and to find a chemo-enzymatic route to the (1*S*,2*R*)-stereoisomer.

2. Experimental

IR spectra were measured on a Jeol FT-IR SPX60 spectrometer. ^{1}H NMR spectra were measured in $CDCl_3$ at 400 MHz on a VARIAN 400-MR spectrometer or at 500 MHz on a VARIAN 500-MR spectrometer. ^{13}C NMR spectra were measured in $CDCl_3$ at 100 MHz on a VARIAN 400-MR spectrometer or at 125 MHz on a VARIAN 500-MR spectrometer. HPLC data were recorded on SHIMADZU SPD-M20A multi-channel detector. Optical rotation values were recorded on a Jasco P-1010 polarimeter. High resolution mass spectra (HRMS) were recorded on JEOL JMS-T100LP AccuTOF. TLC analysis and preparative TLC purification were performed with Merck Silica Gel 60 F_{254} plates (0.25 mm thickness, No. 5715 and 0.5 mm thickness, No. 5744), respectively. Silica gel 60 N (spherical, neutral, 63–210 μm, 37565-84) from Kanto Chemical Co. was used for column chromatography. Peptone and yeast extract were purchased from Kyokuto Pharmaceutical Co., for the cultivation of microorganism. Yeast strains are available at Japan Collection of Microorganisms; Riken Bioresource Center, Planning Section, Research Promotion Division, RIKEN Tsukuba Institute, 3-1-1 Koyadai, Tsukuba, Ibaraki 305-0074, Japan, and to NITE Biological Resource Center; Department of Biotechnology, National Institute of Technology and Evaluation, 2–5-8 Kazusakamatari, Kisarazu-shi, Chiba 292-0818, Japan. Primary kit of Chiralscreen® OH was purchased from Daicel Corp.

2.1. Ethyl (1R*,2R*)- and (1R*,2S*)-2-hydroxycyclohexanecarboxylate (**1a**), and (1R*,2R*)- and (1R*,2S*)-2-hydroxycyclohexanemethanol

To a solution of **2a** (197 mg, 1.16 mmol) in EtOH (6.5 mL) was added $NaBH_4$ (159 mg, 4.21 mmol) at 0 °C. The mixture was stirred for 1 h at that temperature. The reaction was quenched with cold aqueous 10% AcOH solution and the organic materials were extracted with AcOEt. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (750 mg). Elution with hexane/AcOEt (10:1) afforded *syn*-**1a** (12.1 mg, 6.1%) and *anti*-**1a** (20.2 mg, 10.1%) as colorless oil. For (1*R**,2*S**)-**1a** (*syn*-isomer): ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.24-1.36 (m, 5H), 1.39-1.51 (m, 2H), 1.64-1.75 (m, 2H), 1.83-2.01 (m, 2H), 2.47 (ddd, J = 2.7, 3.8, 11.2 Hz, 1H), 4.09-4.21 (m, 3H). For (1*R**,2*R**)-**1a** (*anti*-isomer): ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.18-1.40 (m, 7H), 1.69-1.79 (m, 2H), 1.99-2.07 (m, 2H), 2.24 (ddd, J = 3.7, 9.8, 12.3 Hz, 1H), 3.76 (ddd, J = 4.5, 9.8, 9.8 Hz, 1H), 4.17 (q, J = 7.0 Hz, 2H). There were unidentified signals: δ 3.70-4.04 in the ^{1}H NMR spectrum of crude product. Based on the benzoylation in the next step, those were assumed to be the contaminated diols caused by overreduction.

2.2. Ethyl (1R*,2R*)- and (1R*,2S*)-2-benzoyloxycyclohexanecarboxylate (**3a**), and (1R*,2R*)- and (1R*,2S*)-2-benzoyloxymethylcyclohexyl benzoate

To a solution of **1a** (12.1 mg, 7.03 μmol) in anhydrous pyridine (0.5 mL) was added benzoyl chloride (25 μL, 0.215 mmol) and catalytic amount of 4-(*N*,*N*-dimethylamino)pyridine (DMAP). The mixture was stirred for 4 h at room temperature. The reaction was quenched by the addition of saturated aqueous NH_4Cl solution and the organic materials were extracted with AcOEt. The organic layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was purified by preparative TLC (developed with hexane/AcOEt = 5:1) to give **3a** (8.5 mg, 43.6%) as colorless oil. For (1*R**,2*S**)-**3a** (*syn*-isomer): ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.11 (t, J = 7.1 Hz, 1H), 1.55-1.62 (m, 4H), 1.87-2.04 (m, 3H), 2.15-2.19 (m, 1H), 2.63 (ddd, J = 3.0, 4.6, 11.4 Hz, 1H), 4.00-4.14 (m, 2H), 5.64 (br, 1H), 7.39-7.46 (m, 2H), 7.52-7.57 (m, 1H), 8.00-8.03 (m, 2H). HPLC [column, Daicel CHIRALPAK® ID, 0.46 cm x 25 cm; hexane/*i*-PrOH = 85:15, flow rate 0.5 mL/min, detected at 237 nm], t_R (min) = 13.6, 14.4. In the ^{1}H NMR spectrum and HPLC chart, due to *cis*-diol dibenzoate, extra signals (δ 4.21 (dd, J = 8.2, 11.0 Hz, 1H), 4.34 (dd, J = 6.6 11.0 Hz, 1H), 5.49 (br, 1H)) and an extra inseparable peak (t_R = 18.8) appeared, respectively. Calculated from ^{1}H NMR spectrum, this product was contaminated with *cis*-diol dibenzoate (*ca.* 25%). For (1*R**,2*R**)-**3a** (*anti*-isomer): ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.12 (t, J = 7.1 Hz, 3H), 1.25-1.35 (m, 1H), 1.38-1.53 (m, 2H), 1.57-1.67 (m, 1H), 1.74-1.79 (m, 1H), 1.80-1.84 (m, 1H), 2.03-2.07 (m, 1H), 2.21-2.25 (m, 1H), 2.66 (ddd, J = 3.9, 10.3, 12.0 Hz, 1H), 4.02-4.11 (m, 2H), 5.21 (ddd, J = 4.4, 10.3, 10.3 Hz, 1H), 7.41-7.47 (m, 2H), 7.52-7.56 (m, 1H), 7.99-8.02 (m, 2H). HPLC analysis in the same conditions for *syn*-isomer, t_R (min) = 16.5, 17.9. In the ^{1}H NMR spectrum and HPLC chart, due to *anti*-diol dibenzoate, extra signals (δ 4.25 (dd, J = 6.0, 11.0 Hz, 1H) 4.45 (dd, J = 4.1, 11.0 Hz, 1H), 5.01 (ddd, J = 4.6, 10.1, 10.1 Hz, 1H)) and two peaks (t_R = 18.8, 21.6) appeared, respectively. Calculated from ^{1}H NMR spectrum, this product was contaminated with *anti*-diol dibenzoate (*ca.* 25%). The relationships between the retention time in HPLC analysis of each peak and stereoisomer of **3a** was assigned as follows, taking the results in Sections 2.3, 2.6, and with an authentic sample made by bakers' yeast-catalyzed reduction into account; 13.6 for (1*R*,2*S*)-**3a**, 14.4 for (1*S*,2*R*)-**3a**, 16.5 for (1*S*,2*S*)-**3a**, 17.9 for (1*R*,2*R*)-**3a**.