Tetrahedron: *Asymmetry* 24 (2013) 440–443

Contents lists available at SciVerse ScienceDirect

Tetrahedron: *Asymmetry*

journal homepage: www.elsevier.com/locate/tetasy

Stereoselective bioreduction of β-carboline imines through cell-free extracts from earthworms (*Eisenia foetida*)

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ARTICLE INFO

Article history:
Received 10 January 2013
Accepted 6 March 2013

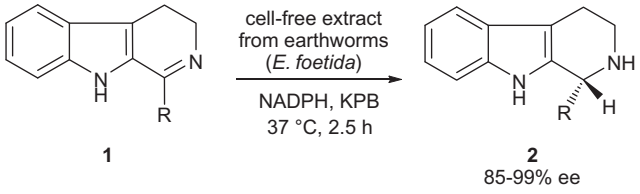
ABSTRACT

Although remarkable advances have been made over the last decade in organic synthesis, catalysis, and biotechnology, there is still a need to introduce and develop new processes for chemical production to achieve sustainable and cleaner approaches to support the increasing global pharmaceutical/chemical industry. There is a growing need to produce optically active compounds in high yields to maintain and support areas such as pharmaceutical and natural product synthesis. Thus, chemists today are looking for alternative reactions carried out under green conditions. In this context, we describe β-carboline imine reductions employing cell-free extracts from red Californian earthworms (*Eisenia foetida*) in high yields and enantiomeric excesses. The enantiomeric excess values of the bioreduction showed no dependence on the imine **1a**–**g** substituents to afford amines with an (*R*)-configuration. Based on these data, a model for the cell-free extract from the earthworm is proposed.

1. Introduction

Biocatalysis[1]has attracted a growing interest in several fields such as genetics,[2] molecular biology,[3] fermentation technology,[4] bioinformatics,[5] nanotechnology,[6] material sciences,[7] advanced spectroscopy,[8] asymmetric organic synthesis,[9] and others.[10] Today, biocatalysis is becoming one of the most powerful green tools in chemistry and biotechnology. Several examples using multienzymatic systems have been demonstrated for the large scale production of pharmaceuticals and fine chemicals.[11] Currently, biocatalysis has started to provide an important tool in synthetic organic chemistry because there is an ever growing need to produce optically active intermediate compounds under green conditions.[12]

The availability of a certain microorganism is often the determining issue for an organic chemist when studying biotransformations in synthetic reactions.[13] For example, Baker's yeast (BY, *Saccharomyces cerevisiae*, and *S. cerevisiae*) is a readily available and often used microorganism.[14,15] For the production of chiral amines from imines, we recently reported the bioreduction of β-carboline imines mediated by *Saccharomyces bayanus*, which gave amine products in good yields and enantiomeric excesses.[9e] Invertebrates, for example, earthworms that are a widespread reptile living in the loose and moist soils, which have been recognized as the main ecosystem engineers,[17,18] have not been used as alternative biocatalysts to date.[16] There are many reports available on anthelmintic activity studies. However, it was only until a few years ago when they started to attract more interest in the area of waste biorecycling/biodegradation.[19–25] Ishihara et al. reported the stereoselective reduction of carbonyl compounds using the cell-free extract from earthworms (*Lumbricus rubellus*) in the presence of NADH or NADPH as a coenzyme.[24] There are a few reports that employ cell-free extracts as biocatalysts to conduct asymmetric reduction of ketones, however there are no reports based on prochiral imines.



Scheme 1. Bioreduction of β-carboline imines by cell-free extract from *E. foetida*.

Since a current challenge in synthetic organic chemistry is chiral induction, we pursued the development of an efficient and green methodology for the stereoselective synthesis of optically active amines **2**, via key intermediate **1** through bioreduction of β-carboline imines employing the cell-free extract from earthworms (*Eisenia foetida*) in the presence of NADPH, as depicted in Scheme 1.

2. Results and discussion

Imine **1** was obtained in 75–83% overall yield from different carboxylic acids and tryptamine by coupling with EDC/HOBt in

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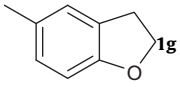
http://dx.doi.org/10.1016/j.tetasy.2013.03.003

CH_2Cl_2 at room temperature, which gave the corresponding amides. The amides were reacted by using Bischler–Napieralsky cyclization conditions to afford imines **1**.[28a] After the production of several imines **1a–g**, we next set out to explore the asymmetric reduction. In nature, oxidoreductases catalyze selective transfer hydrogenations of carbonyl compounds to alcohols using co-factors such as NAD(P)H. Therefore, NADPH might reduce imines in a similar manner. Thus, the scope of the reduction of these imines employing cell-free extracts from earthworms (*E. foetida*) as a biocatalyst was first investigated according to the reaction times (Scheme 1, Table 1).

Table 1
Reduction of imines **1a–g** by cell-free extract from *E. foetida*

Entry	R	ee%[a,b]	Absolute configuration	Yield[b] (%)
1	Me **1a**	>99	(*R*)	80
2	Et **1b**	96	(*R*)	77
3	*iso*-Pr **1c**	96	(*R*)	75
4	*iso*-Bu **1d**	97	(*R*)	83
5	Ph **1e**	92	(*R*)	74
6	$-(CH_2)_{10}CH_3$ **1f**	95	(*R*)	63
7	 **1g**	95	(*R*)	68

[a] Absolute configurations were determined by HPLC employing a ChiralPack-OD column.
[b] No changes were observed in the ee% or yields according to reaction times between 2.5 and 24 h.

The reduction of **1a** was carried out using an aqueous solution of the cell-free extract, NADPH in a potassium phosphate buffer (pH 7), and the mixture was stirred until completion of the reaction, as monitored by TLC at 37 °C.[24] After completion of the reaction, the crude was basified with NaOH (1.0 M) to pH 10, after which brine was added and extracted with Et_2O. Amine **2a** was obtained in 80% yield and >99% ee after 2.5 h. Then, in order to test whether the reaction times might influence the ee% and yields, we performed another experiment of **1a** under the same conditions described above with long reaction times. After 24 h of reaction, the ee% values were similar to those previously as obtained, and no improvement in the yields was observed (Table 1, entry 1). Next, we tested other imines for the reduction with cell-free extracts and NADPH, as depicted in Table 1. Enantiomeric excesses ranging from 92% to 97% ee were observed with moderate to good yields (63–83%) of the amines **2b–g**, as depicted in Table 1 (entries 2–7).

Finally, the absolute configuration of all the amine products was determined to be (*R*); this was corroborated according to those authentic amines obtained previously by the Noyori asymmetric reduction of imines.[26,28]

For **1**, the enzyme has to distinguish between the *Si*- and the *Re*-face of the π-system in order to yield chiral **2**. The asymmetric reduction of imine-containing compounds by free-cell extract/NADPH is not a widespread reaction. According to the results obtained in Table 1, the imine reduction suggested a hydrogen transfer to the *Si*-face of prochiral imine **1** with no influence of the R groups. A noteworthy difference was observed when compared with β-carboline imine reductions mediated by *S. bayanus*.[9e] In this case, hydrogen transfers to the *Re*-face of prochiral imine **1** when R is R_S and hydrogen transfers to the *Si*-face when R is R_L or an aromatic substituent adjacent to the imine group to yield amine **2**, as depicted in Figure 1. However, we noticed that when free-cell extract/NADPH was used, considerably higher ee% and yields were obtained when compared with the whole-cell approaches.[9e] It is known that in whole-cell biocatalysis, the cellular membrane often retards the entry of the substrate into the cellular systems and prevents the product from being released from the cellular system for easy recovery, which explains the better yields observed in the enzyme free-cell extracts.[27]

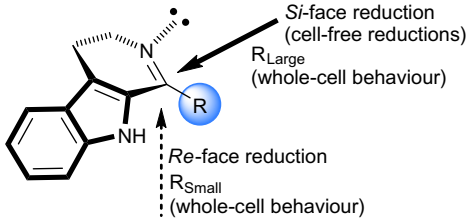


Figure 1. Proposed model for the bioreduction of β-carboline imines by the cell-free extract from *E. foetida* (*Si*-face reductions) and $R_{L/S}$ group dependence found in whole-cell reductions mediated by Saccharomyces.[9e]

3. Conclusion

In conclusion, we have demonstrated the feasibility of this novel protocol for the asymmetric biocatalytic reduction of β-carboline imines to chiral amines through a cell-free extract from red Californian earthworms with yields and %ee comparable to those obtained by Noyori ruthenium methodology. Moreover, comparing our approach with whole-cell systems, the cell-free extract has the advantages of affording higher yields and selectivities, whereas in the whole-cell method, we have observed that the cellular membrane may retard the entry of the substrate into the cellular systems as well as inhibit the product from being released from the system thus giving lower yields. This methodology is an attractive alternative to the catalytic asymmetric methods employing Noyori catalysts as well as whole-cell yeasts.

4. Experimental

4.1. General

4-(2-Aminoethyl) benzenesulfonyl fluoride hydrochloride, trypsin inhibitor from *Glycine max* (soybean), and β-nicotinamide adenine dinucleotide reduced dipotassium salt (β-NADPH) were obtained from Sigma–Aldrich. Imine compounds were synthesized according to previously described protocols.[26] Hexane, 2-propanol, and diethylamine were of HPLC grade and used without further treatment. Benzene, tryptamine, $POCl_3$, $NaBH_4$, as well as the organic acids for imine preparation were purchased from Merck, and used without previous purification. The enantiomeric excess (ee) values of the amine products were determined by HPLC analysis (Agilent 1260) equipped with an optically active capillary column (ChiralPack OD) by using hexane/2-propanol/diethylamine (80:20:0.1) as the mobile phase at λ 254.

4.2. Microorganisms

Red Californian earthworms (*E. foetida*) were collected from an earthworm farm provider from Talca city, Maule, Chile. The samples of earthworm (approximately 25 g, equivalent to 60 earthworms) were mixed with 50 mL of potassium phosphate buffer (KPB, 50 mM, pH 7). This suspension was cooled below 4 °C, and subsequently homogenized in a blender for 1 minute at room temperature. Then, the crude was placed in a refrigerated centrifuge (Velocity 14R, Dynamica) at 10,000 rpm for 30 min at 4 °C, thus eliminating cell debris. 4-(2-Aminoethyl) benzenesulfonyl fluoride hydrochloride (final concentration, 10 mM) and trypsin inhibitor (final concentration, 0.1 mM) were added as protease inhibitors. The mixture was stirred gently for 30 min at 4 °C, and upon completion was again centrifuged at 10,000 rpm for 40 min at 4 °C. The resulting supernatant (24.1 mL) was the crude cell-free extract.[24]

4.3. Bioreduction

In a test tube of 10 mL were placed 1.0 mL of cell-free extract from *E. foetida*, NADPH (11 μmol), imines **a**–**g** (10 μmol), and 0.1 M KPB (pH 7.0). The mixture was placed in an incubator shaker (ZHWY-100B, ZHICHENG) to 200 rpm at 37 °C with constant gentle agitation for 2.5 and 24 h, respectively. After completion of the reaction as determined by TLC monitoring, the mixture was basified with aqueous NaOH solution (1.0 M), extracted with diethyl ether, dried with anhydrous sodium sulfate, and concentrated under reduced pressure to furnish the crude products, which were purified by silica-gel column chromatography, and finally analyzed by HPLC to determine the ee%.

4.3.1. (*R*)-1-Methyl-1,2,3,4-tetrahydro-9*H*-pyrido[3,4-*b*]indole 2a

$[\alpha]_D$ = +52 (*c* 1.0, MeOH), {lit.[28c] (*R*)-isomer, $[\alpha]_D$ = +53.5 (*c* 2.08, EtOH)}, >99% ee by HPLC analysis (Chiralcel OD, hexane/2-propanol/diethylamine = 80:20:0.1, 1.0 mL/min, 254 nm, minor isomer 8.7 min, major isomer 5.6 min). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.46 (d, 3H, *J* = 6.7 Hz), 1.80 (br s, 1H), 2.88–2.83 (m, 2H), 3.05 (ddd, 1H, *J* = 13.1, 9.2, 5.2 Hz), 3.37 (ddd, 1H, *J* = 13.1, 5.2, 3.7 Hz), 4.19 (tq, 1H, *J* = 6.7, 2.0 Hz), 7.09 (dt, 1H, *J* = 7.3, 0.9 Hz), 7.15 (dt, 1H, *J* = 7.3, 0.9 Hz), 7.31 (d, 1H, *J* = 7.3 Hz), 7,48 (d, 1H, *J* = 7.3 Hz), 7.78 (br s, 1H). HRMS (ESI): *m*/*z* calcd for $C_{12}H_{14}N_2$ 187.1235, found 187.1234 $[M+H]^+$. The spectroscopic data are in accordance with previously reported data.[28a]

4.3.2. (*R*)-1-Ethyl-1,2,3,4-tetrahydro-9*H*-pyrido[3,4-*b*]indole 2b

$[\alpha]_D$ = +61.0 (*c* 1.0, MeOH), {lit.[29] (*S*)-isomer, $[\alpha]_D$ = −62.6 (CH_3COCH_3)}, 96% ee by HPLC analysis (ChiralPack OD, hexane/2-propanol/diethylamine = 80:20:0.1, 1.0 mL/min, 254 nm, major isomer 8.8 min, minor isomer 11.2 min). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.10 (t, 3H, *J* = 7.1 Hz), 1.67–1.75 (m, 1H), 1.85–2.07 (m, 1H), 2.72–2.78 (m, 2H), 3.00–3.06 (m, 1H), 3.34–3.40 (m, 1H), 4.00–4.04 (m, 1H), 7.06–7.19 (m, 2H), 7.32 (d, 1H, *J* = 7.2 Hz), 7.49 (d, 1H, *J* = 7.6 Hz), 7.77 (br s, 1H). HRMS (ESI): *m*/*z* calcd for $C_{13}H_{16}N_2$ 201.1392, found 201.1395 $[M+H]^+$. The spectroscopic data are in accordance with previously reported data.[29b–d]

4.3.3. (*R*)-1-Isopropyl-1,2,3,4-tetrahydro-9*H*-pyrido[3,4-*b*]indole 2c

$[\alpha]_D$ = +57.2 (*c* 1.0, MeOH),[30] 96% ee by HPLC analysis (ChiralPack OD, hexane/2-propanol/diethylamine = 80:20:0.1, 0.8 mL/min, 254 nm, major isomer 6.0 min, minor isomer 8.0 min). FT-IR: (KBr film, cm^{-1}) 1466, 3471. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.91 (d, 3H, *J* = 7.2 Hz), 1.14 (d, 3H, *J* = 7.2 Hz), 2.16–2.28 (m, 1H), 2.72–2.77 (m, 2H), 2.93–3.03 (m, 1H), 3.42–3.45 (m, 1H), 4.00–4.04 (m, 1H), 7.10–7.15 (m, 2H), 7.31 (dd, 1H, *J* = 1.4, 7.3 Hz,), 7.50 (dd, 1H, *J* = 1.4, 7.3 Hz,), 7.85 (br s, 1H). HRMS (EI): *m*/*z* calcd for $C_{14}H_{18}N_2$ 214.1466, found 214.1468. The spectroscopic data are in accordance with previously reported data.[30]

4.3.4. (*R*)-1-Isobutyl-1,2,3,4-tetrahydro-9*H*-pyrido[3,4-*b*]indole 2d

$[\alpha]_D$ = +36.1 (*c* 1.0, MeOH), 97% ee. FT-IR: (KBr film, cm^{-1}) 1469, 3480. ^{1}H NMR ($CDCl_3$): δ 1.00 (d, 3H, *J* = 6.4 Hz), 1.03 (d, 3H, *J* = 6.4 Hz), 1.62 (m, 2H), 1.87–2.04 (m, 2H), 2.74 (m, 2H), 3.03 (ddd, 1H, *J* = 5.4, 7.9, 13.3 Hz), 3.35 (dt, 1H, *J* = 4.6, 12.9 Hz), 4.11 (m, 1H), 7.09 (ddd, 1H, *J* = 1.2, 6.1, 8.3 Hz), 7.14 (ddd, 1H, *J* = 1.2, 7.6, 8.8 Hz), 7.30 (d, 1H, *J* = 7.9 Hz), 7.48 (d, 1H, *J* = 7.6 Hz), 7.73 (br s, 1H). HRMS (ESI): *m*/*z* calcd for $C_{15}H_{20}N_2$ 229.1705, found 229.1700 $[C_{15}H_{20}N_2+H]^+$. The spectroscopic data are in accordance with previously reported data.[28b,c]

4.3.5. (*R*)-1-Phenyl-1,2,3,4-tetrahydro-9*H*-pyrido[3,4-*b*]indole 2e

$[\alpha]_D$ = −4.2 (*c* 1.0, $CHCl_3$), {lit. (*R*)-isomer, $[\alpha]_D$ = −3.9 (*c* 1.03 in $CHCl_3$)},[28c] 92% ee by HPLC analysis (ChiralPack OD, hexane/2-propanol/diethylamine = 80:20:0.1, 1.0 mL/min, 254 nm, minor isomer 15.8 min, major isomer 19.9 min). ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.83–2.95 (m, 2H), 3.07–3.16 (m, 1H), 3.33–3.39 (m, 1H), 5.17 (s, 1H), 7.10–7.19 (m, 3H), 7.30–7.37 (m, 5H), 7.56–7.60 (m, 1H), 7.66 (br s, 1H). HRMS (EI): *m*/*z* calcd for $C_{17}H_{16}N_2$ 248.1314, found 248.1309. The spectroscopic data are in accordance with previously reported data.[28c]

4.3.6. (*R*)-1-Undecyl-1,2,3,4-tetrahydro-9*H*-pyrido[3,4-*b*]indole 2f

Compound **2f** has not previously been described: $[\alpha]_D$ = +52.4 (*c* 1.0, MeOH), 95% ee by HPLC analysis (ChiralPack OD, hexane/2-propanol/diethylamine = 80:20:0.1, 0.8 mL/min, 254 nm, major isomer 7.35 min, minor isomer 9.9 min). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.80 (t, 3H, *J* = 7.0 Hz), 1.10–1.89 (m, 16H), 1.30–1.45 (m, 2H), 1.59–1.69 (m, 1H), 1.72–1.82 (m, 1H), 2.62–2.73 (m, 2H), 2.90–3.00 (m, 1H), 3.20–3.30 (m, 1H), 4.00 (br s, 1H), 7.03 (t, 1H, *J* = 7.0 Hz), 7.09 (t, 1H, *J* = 7.0 Hz), 7.24 (d, 1H, *J* = 7.9 Hz), 7.39 (d, 1H, *J* = 7.9 Hz), 8.00 (br s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.1, 22.7, 25.9, 29.4, 29.7, 29.73, 29.75, 29.79, 29.9, 32.0, 34.6, 36.0, 41.7, 52.4, 108.4, 110.9, 118.1, 119.4, 121.7, 127.3, 135.0, 135.8. HRMS (ESI): *m*/*z* calcd for $C_{22}H_{34}N_2$ 327.2802, found 327.2809 $[C_{22}H_{34}N_2+H]^+$.

4.3.7. (*R*)-1-(2,3-Dihydro-5-benzofuranyl)-2,3,4,9-tetrahydro-1*H*-β-carboline 2g

$[\alpha]_D$ = +22.3 (*c* 1.0, MeOH). ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.60–3.02 (m, 2H), 3.07 (t, 2H, *J* = 8.2 Hz), 3.05–3.20 (m, 1H), 3.26–3.45 (m, 2H), 4.50 (t, 2H, *J* = 8.2 Hz), 5.16 (s, 1H), 6.74 (d, 1H, *J* = 7.8 Hz), 6.97–7.29 (m, 5H), 7.54 (d, 1H, *J* = 7.8 Hz), 10.4 (s, 1H). The spectroscopic data are in accordance with previously reported data.[26b]

Acknowledgments

The authors are thankful to the agronomist Camilo A. Rosales Godoy for providing red Californian earthworms (*E. foetida*). This work has been granted by FONDECYT (1110022). L.S.S. thanks Proyecto Anillo (Integración de la Biología Estructural al desarrollo de la Bionanotecnología, ACT 1107) for the support of research activities.

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