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Synthesis of S-licarbazepine by asymmetric reduction of oxcarbazepine with *Saccharomyces cerevisiae* CGMCC No. 2266

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ABSTRACT

S-licarbazepine was synthesized by asymmetric reduction of oxcarbazepine with CGMCC No. 2266. The optimum batch reduction conditions were found to consist of a reaction time of 36 h, temperature of 30 °C, and initial pH value of 7.0. The optimum concentration of the glucose co-substrate was found to be 0.3 mol L^{-1}. The addition of glucose contributed to *in situ* regeneration of NADPH in cells and improved conversion. Conversion increased with the addition of more biomass and with a decrease in the initial concentration of substrate. Within the membrane reactor, a continuous reduction process was used to improve production efficiency and reduce the inhibition of high-concentration substrate upon reduction. The optimum flux was found to be 20 ml h^{-1}. S-licarbazepine yield was 3.7678 mmol L^{-1} d^{-1} in continuous reduction over four days. The enantiometric excess of S-licarbazepine was 100% for both batch and continuous reduction processes.

1. Introduction

S-licarbazepine (S-LC) can be used in synthesis of eslicarbazepine acetate by esterification with acetic acid. Eslicarbazepine acetate [S-(−)-10-acetoxy-10,11-dihydro-5H-dibenz/-b,f/azepine-5-car-Boxamide, ESL], formerly known as BIA 2-093, is a novel central nervous system (CNS)-active compound with anticonvulsant activity [1–5]. It behaves as a voltage-gated sodium channel blocker and is currently under clinical development for the treatment of epilepsy and bipolar disorder [6–8]. In humans, ESL is more rapidly reduced by liver esterases to the major active metabolite S-licarbazepine than oxcarbazepine (OXC), a new antiepileptic drug approved for the treatment of partial onset seizures and generalized tonic-clonic seizures [9–18].

This paper discusses in detail the synthesis of S-licarbazepine by asymmetric reduction of oxcarbazepine with CGMCC No. 2266 as a catalyst. This is the first report on biosynthesis of S-licarbazepine using microorganism catalysts. Industry has adopted chemical synthesis as a preferred method of production of S-licarbazepine. No references exist on the enzymatic asymmetric reduction of OXC to S-LC. S-licarbazepine can be obtained by asymmetric reduction of oxcarbazepine within microorganisms (Fig. 1). Chiral alcohol, an intermediate of many different pharmaceuticals, can be obtained by asymmetric reduction of prochiral ketones through biotransformation [19–22]. Asymmetric reduction with microorganism catalysts has many advantages for the production of chiral compounds. These include safety and reliability, low cost, scalability, a lack of environmentally dangerous by-products, the fact that cell culture facilities already exist in many laboratories, and the purity of the final chiral product. In addition, the regeneration of coenzyme (NADH and NADPH) *in situ* can improve conversion because of many kinds of enzyme exist in cells [23,24].

2. Experimental procedure

2.1. Reagents and instruments

Oxcarbazepine and S-licarbazepine were purchased from Zhejiang Jiuzhou Pharma Science & Technology Co., Ltd. (China). Agilent HPLC 1200 equipped with Daicel OD-H chiral column from Daicel Company was used in detection of substrate and product content and enantiometric excess of S-licarbazepine. The membrane reactor and ultrafiltration membrane (MWCO 30 kDa) were from Shanghai Mosu Company (China).

2.2. Microorganism cultivation

Saccharomyces cerevisiae CGMCC No. 2266 was obtained from the soil in the vicinity of Hang Zhou West Lake Brewery and preserved in China General Microbiology Culture Collection Cen-

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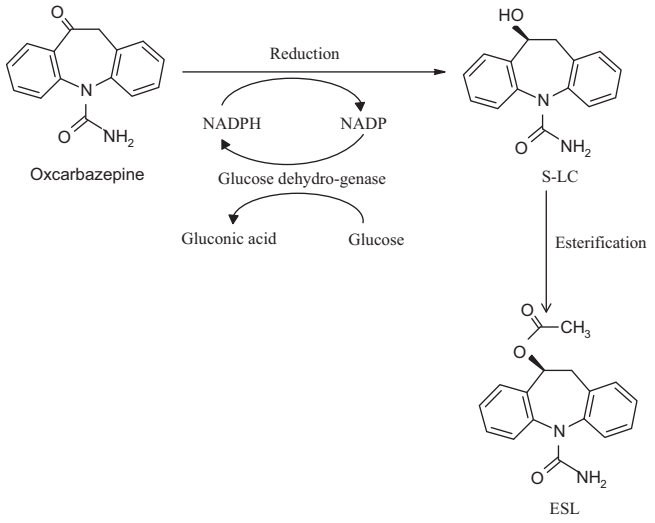

Fig. 1. Synthesis of ESL by asymmetric reduction of OXC.

ter on November 26, 2007. Slant medium for strain storage was composed of 10 g L^{-1} malt juice, 3 g L^{-1} yeast extract, 5 g L^{-1} peptone, 10 g L^{-1} glucose and 20 g L^{-1} agar. The liquid yeast culture medium was composed of 30 g L^{-1} glucose, 3 g L^{-1} yeast extract, 5 g L^{-1} $(NH_4)_2SO_4$, 0.25 g L^{-1} $MgSO_4$, 1 g L^{-1} $K_2HPO_4{\cdot}3H_2O$, and 1 g L^{-1} KH_2PO_4. The strain picked from slant medium was inoculated into 100 ml liquid culture medium and cultivated in 30 °C shaker (200 r min^{-1}) for 24 h. Then 10 ml of the cell suspension was inoculated into 100 ml liquid medium. After 24 h of cultivation, the cells were harvested and used in reduction.

2.3. Preparation of oxcarbazepine alcohol solution

Oxcarbazepine alcohol solution was prepared by the addition of oxcarbazepine to hot alcohol, which was then cooled to room temperature. The concentration of the oxcarbazepine alcohol solutions were 0.793, 1.982, 3.964, 5.987, and 7.928 mmol L^{-1}.

2.4. Batch reduction

The cultivated cells were separated from the culture medium by centrifugation. The cells from the sediment were washed twice with sterile water and separated by centrifugation before being in reduction. The sediment obtained above and 10 ml oxcarbazepine alcohol solution were added to a flask containing 100 ml of 0.2 mol L^{-1} phosphate buffered solution for reduction. The reaction was stopped by centrifugation at the end of the reduction period. The supernatant obtained above was extracted by ethyl acetate. The ethyl acetate layer was detected for determination of the substrate and product content by Agilent HPLC 1200.

2.5. Continuous reduction

Fig. 2 shows the continuous reduction of oxcarbazepine in the membrane reactor. One hundred milliliters of oxcarbazepine alcohol solution was pumped from vessel 1 into a continuously operated stirred membrane reactor (reactor 2). The flow rate of oxcarbazepine alcohol solution was 10–50 ml h^{-1}. The 200 ml of reduction mixture in reactor 2 was composed of cells, 0.3 mol L^{-1} glucose, and phosphate buffer (pH 8.0). The cell dry weight in the mixture was 28 g. The cells were trapped by an ultrafiltration membrane (MWCO 30 kDa). The membrane was precoated with 1 mg of bovine serum albumin per ml reactor volume to prevent cell adsorption. The whole reactor was sterilized with 0.01% peracetic

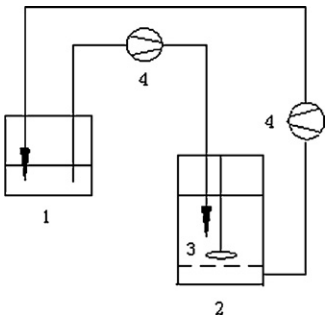

Fig. 2. Continuous reduction process. (1) Substrate alcohol solution storage tank; (2) membrane reactor for continuous reduction; (3) ultrafiltration membrane (MWCO 30 kDa); and (4) peristaltic pump.

acid before use. Percolating fluid from reactor 2 was pumped into vessel 1 for cyclic utilization. Reaction volume, reaction temperature (28 °C), and stirring speed (160 r min^{-1}) remained unchanged. The flux pumped into vessel 1 was equal to that pumped out of vessel 1. At the end of reduction, the liquid in vessel 1 was extracted by ethyl acetate and the ethyl acetate layer was detected for determination of the substrate and product content by Agilent HPLC 1200.

2.6. Production of S-licarbazepine by continuous reduction

After 7.928 mmol L^{-1} of OXC dissolved in 100 ml alcohol solution was completely converted to S-LC, another 100 ml alcohol solution containing 7.928 mmol L^{-1} of OXC was pumped into vessel 2 for continuous reduction. The flux was 20 ml h^{-1}. The process was repeated until the cells in vessel 2 lost reduction activity.

3. Results and discussion

3.1. Influence of initial substrate concentration and reaction time on batch reduction

Fig. 3 shows that conversion increased gradually with reaction time and the rate of increase was very minor after 36 h. The optimum reaction time is therefore 36 h. Conversion decreased with as the concentration of initial substrate increased. This was probably because the reduction activity of cell was inhibited by high con-

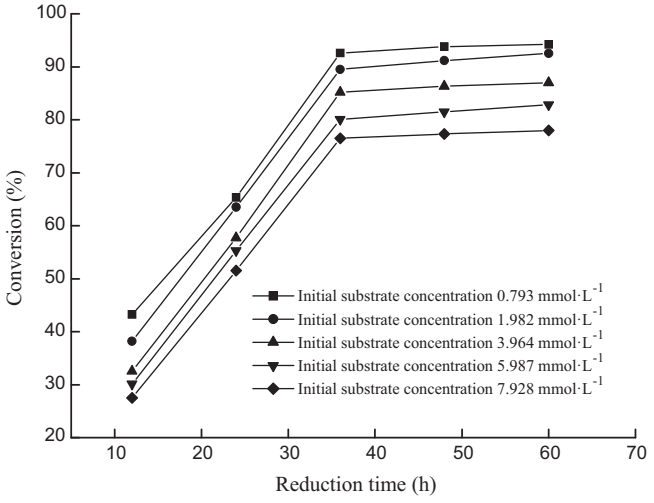

Fig. 3. Influence of initial substrate concentration and reaction time on conversion 30 °C, initial pH value of reaction mixture 7.0, 160 r min^{-1}, cell concentration 140 g L^{-1}.

Table 1
Effect of addition of glucose as co-substrate on conversion.

	Glucose concentration (mol L^{-1})					
	0	0.1	0.2	0.3	0.4	0.5
Conversion (%)	76.5	85.9	90.2	95.7	88.2	78.5

Initial substrate concentration 7.928 mmol L^{-1}, 30 °C, initial pH value of reaction mixture 7.0, 160 r min^{-1}, 36 h, cell concentration 140 g L^{-1}.

Table 2
Effect of initial pH value of reaction mixture on reduction.

	Initial pH value of reaction mixture						
	4	5	6	7	8	9	10
Conversion (%)	75.2	83.8	90.2	95.7	92.5	87.2	72.5

Initial substrate concentration 7.928 mmol L^{-1}, 30 °C, 160 r min^{-1}, 36 h, glucose concentration 0.3 mol L^{-1}, cell concentration 140 g L^{-1}.

Table 3
Effect of temperature on reduction.

	Temperature (°C)								
	10	15	20	25	30	35	40	45	50
Conversion (%)	73.1	75.3	81.2	90.2	95.7	91.2	85.2	80.1	75.2

Initial substrate concentration 7.928 mmol L^{-1}, initial pH value of reaction mixture 7.0, 160 r min^{-1}, 36 h, glucose concentration 0.3 mol L^{-1}, cell concentration 140 g L^{-1}.

centrations of substrate. The enantiometric excess of S-LC achieved 100% and was unaffected by the increase of initial substrate concentration.

3.2. Effects of addition of glucose as co-substrate on reduction

Glucose was selected as co-substrate to improve the conversion. Table 1 shows that glucose helped to improve the conversion and that the optimum glucose concentration was 0.3 mol L^{-1}. Glucose dehydrogenase in cells can convert glucose and NADP to gluconic acid and NADPH, as shown in Fig. 1. NADPH can serve as a hydrogen donor and deliver H^- to OXC to form S-LC. The addition of glucose contributed to the regeneration of NADPH and improved conversion. The enantiometric excess of S-LC was unaffected by the addition of glucose and reached 100%.

3.3. Effect of temperature and initial pH value on reduction

Tables 2 and 3 show the optimum temperature and initial pH value of mixture to be conducive to high conversion rates. The initial pH value of the mixture was adjusted by the addition of 0.2 mol L^{-1} of HCl and 0.2 mol L^{-1} of NaOH. The optimum temperature was found to be 30 °C. The optimum initial pH value of mixture was 7.0. The enantiometric excess of S-LC held steady at 100% under these conditions.

3.4. Effect of cell concentration on reduction

Fig. 4 shows that conversion increased with the concentration of cells used in reduction. Higher conversion rates can be obtained with more biomass because of the large amounts of reductase and co-enzyme NADPH in these cells. The optimum cell concentration was found to be 140 g L^{-1} (cell dry weight/mixture volume). The mixture was too thick to be stirred well when the cell concentration was higher than 140 g L^{-1}. The enantiometric excess of S-LC held steady at 100% and did not vary with cell concentration.

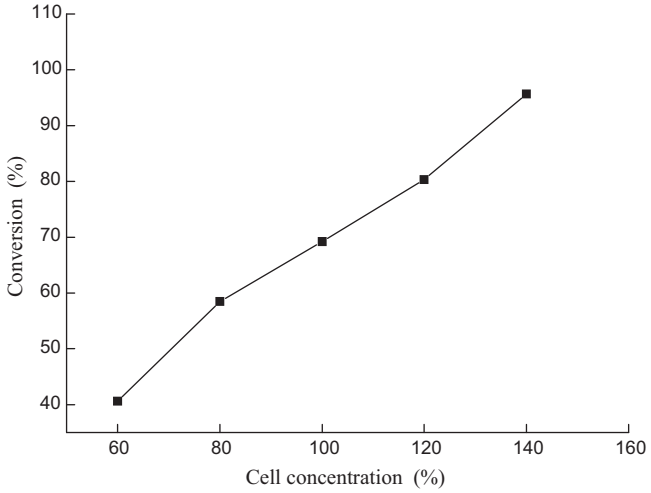

Fig. 4. Effect of cell concentration on reduction. Initial substrate concentration 7.928 mmol L^{-1}, 30 °C, initial pH value of reaction mixture 7.0, 160 r min^{-1}, 36 h, glucose concentration 0.3 mol L^{-1}.

3.5. Effect of flux on continuous reduction

7.928 mmol L^{-1} of OXC dissolved in 100 ml alcohol solution was completely converted to S-LC over 26 h of continuous reduction at flux of 20 ml h^{-1}. 7.928 mmol L^{-1} OXC in 10 ml alcohol solution could not be completely converted to S-LC even after 36 h of batch reduction. This showed continuous reduction to be more efficient than batch reduction. The reduction activity of cells was inhibited by high concentrations of substrate and product in batch reduction. Continuous reduction did not encounter this problem because the substrate and product were continuously pumped out of vessel 2. Fig. 5 shows that the optimum flux in vessel 2 was 20 ml h^{-1}. Complete transformation of substrate was found to take longer when flux was much higher or lower than 20 ml h^{-1}. The enantiometric excess of S-LC held steady at 100% and did not vary by flux.

3.6. Production of S-LC by continuous reduction for reuse of cells

Fig. 6 shows that cells used in continuous reduction processes can be reused three times. Conversion reached 100% in the first, second and third reductions and 80.2% in the fourth. This illus-

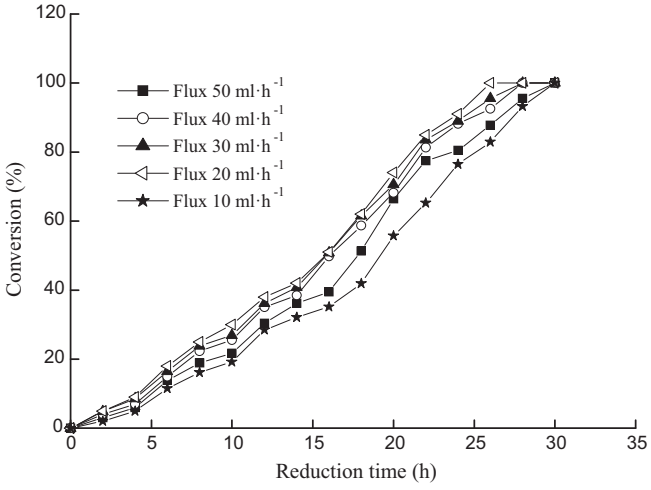

Fig. 5. Effect of flux on continuous reduction. Initial substrate concentration 7.928 mmol L^{-1}, 30 °C, initial pH value of reaction mixture 7.0, 160 r min^{-1}, glucose concentration 0.3 mol L^{-1}.

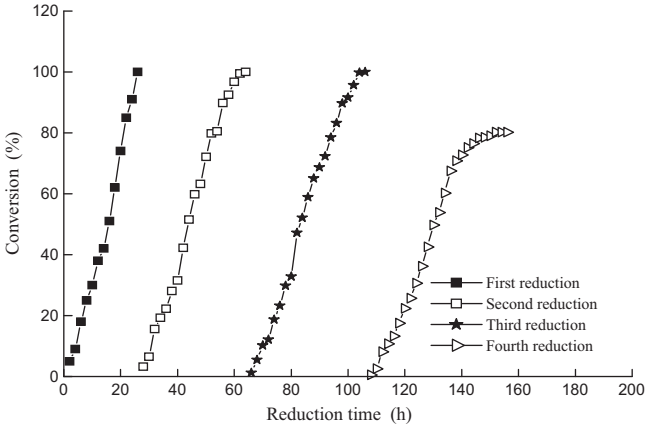

Fig. 6. Production of ESL by continuous reduction with cell reuse.

trates that the cells can be reused reasonably reliably for about 152 h. S-licarbazepine yield was 3.7678 mmol L^{-1} d^{-1} in continuous reduction over four days. The enantiometric excess of ESL was 100% in continuous reduction process.

4. Conclusions

S-licarbazepine was synthesized by batch and continuous reduction of oxcarbazepine with CGMCC No. 2266. The optimum reaction time and temperature were found to be 36 h and 30 °C. The optimum initial reaction mixture pH was found to be 7.0. Conversion increased with increased reaction time and the addition more biomass. Conversion decreased with the addition more substrate. In order to neutralize the inhibitive effect of high concentrations of substrate, a continuous reduction process was used to produce S-LC continuously. The S-licarbazepine yield was 3.7678 mmol L^{-1} d^{-1} after four days of continuous reaction. The enantiometric excess of S-LC was 100% in both batch and continuous reduction process.

Acknowledgements

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