Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

Vol 4, Issue 2, 2012 ISSN- 0975-1491

Research Article

MICROBIAL PRODUCTION OF HYDROCORTISONE BY 11-β-HYDROXYLATION OF CORTEXOLONE BY ASPERGILLUS OCHRACEUS

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Received: 25 Nov 2011, Revised and Accepted: 18 Jan 2012

ABSTRACT

Since the observation by Hench in 1949 of a dramatic response to cortisone in patients with rheumatoid arthritis, corticosteroids have been widely used in medicine. Cortexolone is a suitable precursor for preparing hydrocortisone. In this report 11-hydroxylation of cortexolone by *Aspergillus ochraceus* PTCC 5066 is reported. Seperation and purification of steroid metabolites were performed by preparative thin layer chromatography. Results of spectral analysis of the major metabolite were identical to hydrocortisone.

Keywords: Cortexolone, Hydrocortisone, Biotransformation, Aspergillus ochraceus.

INTRODUCTION

Microbial bioconversion has replaced complex synthetic chemical routes for the stereochemical introduction of oxygen into substrates in the large-scale commercial production of steroids because of the high cost and low yield of chemical synthesis of oxygenated steroids and high regio- and stereospecificity of microbial enzyme systems[1]. Among microbial transformation of steroids, 11-oxygenation of Reichstein's Substance S (cortexolone) belongs to the most important ones. It's a way of producing hydrocortisone which, apart from being a finished medicinal steroid, is also the starting material for the manufacture of several other potent steroids of prednisolone structure[2],a structure that causes no drug-induced salt retention at therapeutic dose levels[3]. The only structural difference between cortexolone and hydrocortisone is the absence of 11-β-hydroxy group in cortexolone (Fig.1). The filamentus fungus *Aspergillus ochraceus* is known as a steroid 11-hydroxylating microorganism and many hydroxylations such as those of androstanes and pregnanes at C-11 by *Aspergillus ochraceus* in which the site of oxygenation is largely independent of substrate functional groups, have been reported previously[4].

In this research we have studied *Aspergillus ochraceus* metabolite while using cortexolone as the substrate. 11β-hydroxylating ability of cortexolone by *Aspergillus ochraceus* PTCC 5060 is reported for the first time.

MATERIALS & METHODS

Strains and growth condition

Aspergillus ochraceus PTCC 5060 was obtained from the Persian Type Culture Collection (Iran). The fungus was maintained on nutrient agar slants. Agar slants were incubated for five days at 25°C to get to desired growth and used to inoculate the precultures. Stock cultures were stored at 4°C and subcultured at monthly intervals.

Steroids and culture mediums

Reichstein's substance S (cortexolone) and hydrocortisone were purchased from Sigma Chemical Co. Nutrient broth and nutrient agar were obtained from Merck Co.

Bioconversion of Reichstein's substance S (cortexolone)

Mature nutrient agar slants were used to inoculate 500 ml culture flasks containing 100 ml of preculture medium consisting of 1.3% nutrient broth (pH=6 after sterilization). The culture was shaken on a shaker incubator at 130 rpm and 30°C for 48 h.

10 ml of this preculture was used to inoculate 100 ml of the production medium. After getting to desired biomass which takes 48 hours, 0.08% cortexolone was added as an ethanolic suspension (final concentration of organic solvent < 1% v/v). A major problem encountered in the fermentation process is low solubility of the substrate and the biotransformation products of this reaction in aqueous media. Cosolvents such as alcohols have been used to enhance the solubility of the steroids[5]. The incubation on the shaker incubator was continued until the production of a sufficient amount of the desired product (see results).

Recovery and purification of biotransformation product

At the end of the transformation period, samples were extracted three times with chloroform. The organic phase was washed with distilled water and then dehydrated over anhydrous sodium sulfate, filtered and dried using rotary evaporator at 50° C. The residue was dissolved in a definite volume of chloroform and applied on TLC plates coated with silicagel GF 254 (thickness 0.5mm). A fresh mixture of chloroform- benzene- methanol- water (90: 5: 4.5: 0.5) was used as the mobile phase. Spots were visualized under ultraviolet light at 254 nm or by spraying with a sulfuric acid- ethanol (4:1) solution, followed by heating at 100° C for about 10 min. Then stamped and eluted with chloroform- methanol (80:20). One of the major metabolites was chosen for spectrophotometrical analysis.

Structure determination of microbial metabolite

The structure of cortexolone metabolite was determined by melting point obtained by Electrothermal, IR obtained by Perkin Elmer 843, ^{13}C NMR and H NMR obtained by NMR 500 Bruker and MASS spectrometry obtained by Finnigan Mat TSQ-70.

Optimization of the incubation time

The optimum incubation period was determined using seven different experiments. The incubation was terminated at 6, 24, 48, 72, 96, 120 and 144 hours, respectively. The biotransformation product was studied by TLC.

The effect of inducer

Different concentrations of cortexolone, in the range of 0.01 to 0.2 g/l, were added to the preculture medium at the beginning of incubation to examine the effect of inducer on biotransformation product.

RESULTS AND DISCUSSION

After separation and purification, the major metabolite was isolated. The physicochemical properties of the major metabolite are described below:

- Melting point: 211-215°C
- Mass spectrometry (EI): MS m/z 362 (M+, $C_{21}H_{30}O_5$), 303 (M-$COCH_2OH$), 285 (M-$COCH_2OH$-H_2O), 227,163,123.
- HNMR (500 MHz, $CDCl_3$): δ 5.69 (s, 1H, H_4), δ 4.49(d, 1H, $OH_{11\beta}$), δ 4.29 (d, 1H, H_{21}), δ 4.64 (d, 1H, H_{21})
- 13CNMR (125 MHz, $CDCl_3$) 211.6(C_{20}), 198.9(C_3), 171.2(C_5), 122(C_4), 88.2, 67.8, 67 (C_{21},C_{17},C_{11}),

- Infrared spectroscopy (IR, $CHCl_3$): max 3400 (s, O-H), 1730 (s, C=O), 1650 (s, C=O)

Spectral and chromatographic data along with the melting point of this compound were identical with the authentic sample of hydrocortisone.

Incubation time: The best time for incubation was estimated to be 96 hours after adding the substrate.

Inducer: The 11-hydroxylase of *A.ochraceus* is inducible and optimal induction occurs at 0.1g/l of cortexolone. The hydroxylase activity increased with increasing concentration of inducer and leveled off at 0.1 g/l.

It has been shown in this study that *Aspergillus ochraceus* has the ability to hydroxylate cortexolone at C-11-β position.

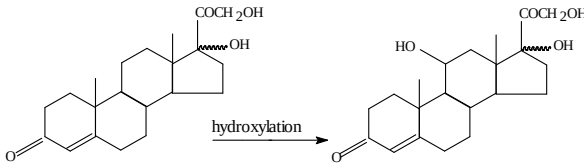

Fig. 1: Microbial conversion of cortexolone to hydrocortisone

ACKNOWLEDGEMENT

The authors would like to thank the Research Council of Shahid Beheshti University of Medical Sciences for financial support of this study. We would like also thank Ms. Soraya Saremy for her kind cooperation in the laboratory.

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