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Biotransformation of Dehydroepiandrosterone by Aspergillus Species and A Comparison of the Results with Previous Studies

Raghda Mahmood Abdulghafoor Al-ani*
Master of Biochemistry, Sakarya University, Institute of Natural Science -Turkey
Email: raghda.alani996@gmail.com

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Abstract

Steroids are widespread in nature, being found in animals, plants, fungi, and certain bacteria. Microbial transformation found wide applications in biotransformation of steroids in order to obtain stereoselective products. This work will explain how to incubate DHEA alongside Aspergillus glaucus MRC 200914 in order to understand the way of this fungus metabolizes the substrate. The main outcome of incubating DHEA with Aspergillus glaucus in this study was the production of several hydroxylated metabolites.

Keywords: Biotransformation; Microbial transformation; Steroids; Dehydroepiandrosterone; Aspergillus; Aspergillus glaucus.

1. Introduction

Biotransformation refers to the use of biological systems to induce chemical changes in compounds that are not their natural substrates (Selamu, 2015).

The microbial transformation of steroids is the most effective use of biotransformation for synthesizing various bioactive compounds (Hassaan, 2024).

Steroids, derived from the Greek word stereos meaning "solids," are solid alcohols found in various animals and plants (SYED ADNAN ALI SHAH, 2013).

The fundamental structure of all steroids is the same: cyclopentanoperhydrophenanthrene, which is made up of four fused rings (Singh, 2017).

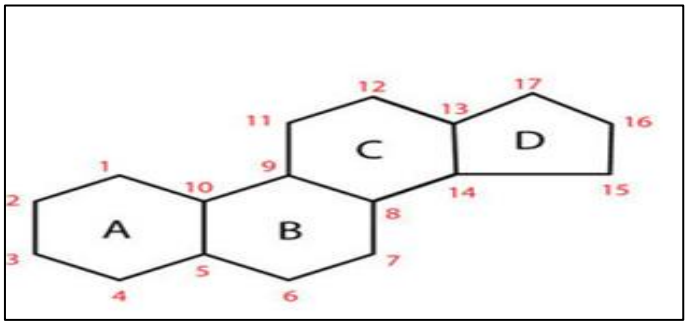

Figure-1. cyclopentanoperhydrophenanthrene (Ronda and Greaves, 2014).

Microbial steroid biotransformation has been known to have potential for several decades, and its use offers several benefits over chemical synthesis. 1) stereospecific or regional functionalization of molecules at locations that chemical agents don't always have access to. 2) a single operation step that involves several successive reactions. 3) environmentally friendly methods such as aqueous media and mild reaction conditions (Lorena, 2018).

*Corresponding Author

The adrenal gland can be considered to primarily produce the steroid dehydroepiandrosterone (DHEA) (Jennifer, 2013).

Among the important and well-known types of steroids, dehydroepiandrosterone is considered one of the most important types. Hydroxylation can be observed at different positions in different biological activities. The hydroxylation at the 11α position is important in the anti-inflammatory activities of prednisolone. As for the hydroxylation at the 14α position, it is considered essential for achieving the production of the 21-acetyl analogue of proligesone, which is known to be a known drug for progesterone in the early stages. Previous biotransformation studies of steroid analogues of dehydroepiandrosterone have shown a diverse spectrum of metabolites (Ming, 2023).

Fungi are widely used in research on microbial steroid conversion. Their diverse set of multifunctional enzymes can convert a large number of different types of steroids (Biotransformation of Progesterone by Whole Cells of Filamentous Fungi Aspergillus brasiliensis, Ming, 2015).

Fungi of all kinds represent an important resource for drug discovery due to their diverse range of medicinal properties, particularly their anticancer and antioxidant activities (Himanshu, 2024).

The genus Aspergillus is highly diverse and has a significant impact on both our economy and society. Species of Aspergillus can be found in various habitats around the world and are often reported as pathogens that affect both humans and animals. These fungi are also known to produce mycotoxins, which can contribute to food spoilage (Samson, 2014).

The genus Aspergillus is particularly noteworthy in biological research due to its capacity to produce a wide range of secondary metabolites (Himanshu, 2024).

The production of six metabolites was achieved as a result of incubation of DHEA with Aspergillus candidus MRC 200634 for continues 5 days (Kudret., 2015).

Dehydroepiandrosterone was hydroxylated at C-6β, C-7β, C-7α, C-11α and C-15α (Kudret, 2015).

Incubation of dehydroepiandrosterone with Aspergillus sydowii MRC 200653 for 5 days yielded three metabolites (Kudret, 2016).

Biotransformations of dehydroepiandrosterone by Aspergillus wentii MRC 200316 for 5 days afforded two metabolites (Kudret, 2012).

Aspergillus glaucus is a resilient xerophilic fungus capable of thriving in various environments due to its physiological adaptations. This fungus may present a mild pathogenic risk to humans (Vit Hubka, 2014).

In this work, the biotransformations of dehydroepiandrosterone by Aspergillus glaucus MRC 200914, which has never been applied in steroid biotransformation before.

2. Material and Methods

The liquid medium for fungus contained 200 g glucose, 5g peptone, 3g malt extract and 3g yeast extract in 1 L of distilled water. The medium was evenly distributed into ten 250 mL Erlenmeyer flasks and sterilized using an autoclave at 121 ºC for 20 minutes. The fungus was then inoculated into these flasks. The flasks were incubated for three days at 25 ºC with shaking at 150 rpm. The flasks were incubated for three days at 25 ºC with shaking at 150 rpm, 1g dehydroepiandrosterone dissolved in 10 mL of DMF was added aseptically to each flask. All flasks were incubated for an additional 5 days under the same conditions. After the incubation period, the mycelium was separated from the broth through vacuum filtration. The mycelium was rinsed with 500 ml of ethyl acetate, and the broth was extracted three times with 1 liter of ethyl acetate. The ethyl acetate extracts were then dried over anhydrous sodium sulfate. The solvent was evaporated under a vacuum to yield a brown gum. Subsequently, chromatography was performed on silica gel using increasing concentrations of ethyl acetate in hexane as the solvent. The results of the incubation and the monitoring of the column chromatography were assessed using 0.2 mm thick TLC plates, with ethyl acetate and n-hexane (1:1, v/v) as the solvent. To develop the chromatograms, the TLC plates were dipped in anisaldehyde-H2SO4 reagent and heated at 120 °C for 3 minutes. A control flask containing sterile uninoculated medium and substrate was carried out for biotransformation experiments. TLC harvested and analyzed the control sample. No metabolites were detected in the control sample. Infrared spectra were recorded. 1H NMR spectra were recorded in CDCl3 at 300 MHz. 13C NMR spectra were recorded in CDCl3 at 75 MHz. Melting points were determined.

3. Results

After incubation of DHEA (1) with Aspergillus glaucus MRC 200914 for five consecutive days, a portion of the initial material (122 mg) was obtained, along with two metabolites: 3β,11α-dihydroxyandrost-5-en-17-one (2) and 3β,7α-dihydroxyandrost-5-en-17-one (3).

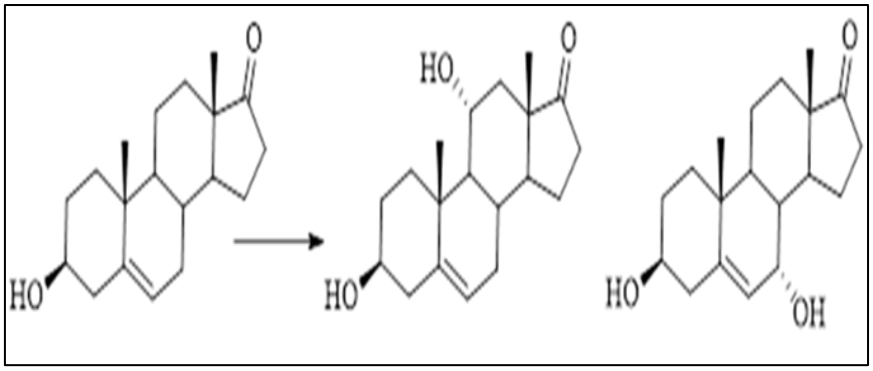

Figure-2. Dehydroepiandrosterone and Metabolites.

When the elution was mixed with 70% ethyl acetate in n-hexane, this resulted in obtaining 3β,11α-dihydroxyandrost-5-ene-17-one (338 mg, 32%), at a melting point of 213-214 °C, lit., 205-206 °C. IR (vmax/cm-1): 3450, 1740, 1650. 1H NMR (300 MHz, CDCl3): 0.87 (3H, s, 18-H), 1.20 (3H, s, 19-H), 3.54 (1H, tt, J = 5 and 10 Hz, 3α-H), 4.08 (1H, dt, J = 5 and 10 Hz, 11β-H), 5.42 (1H, d, J = 5 Hz, 6-H), 13C NMR (75 MHz, CDCl3): (Table 1). Also, by using pure ethyl acetate, the eluent was obtained, as a result of which 317 mg (30%) of 3β,7α-hydroxyandrost-5-ene-17-one was obtained. When the melting point is 181-182 °C, illuminated, 181-182 °C. Infrared (max. speed/cm-1): 3420, 1735, 1655. 1H NMR (300 MHz, CDCl3): 0.90 (3H, s, 18-H), 1.03 (3H, s, 19-H), 3.54 (1H, tt, J = 5 and 10 Hz, 3α-H), 3.97 (1H, bs, 7β-H), 5.60 (1H, d, J = 5 Hz, 6-H). 13C NMR (75 MHz, CDCl3): (Table 1).

Table-1. 13C NMR detail for DHEA, plus metabolites.

C atoms	One	Two	Three
1	37.11	38.93	36.85
2	31.47	31.68	30.94
3	71.48	71.67	71.00
4	42.11	42.54	42.46
5	140.98	141.49	146.38
6	120.83	120.71	123.45
7	31.35	31.33	64.16
8	31.41	30.70	37.41
9	50.13	56.87	41.82
10	36.56	38.28	37.08
11	20.29	68.65	19.97
12	30.71	42.64	31.14
13	47.49	47.96	47.06
14	51.67	50.63	44.82
15	21.82	21.76	21.83
16	35.80	35.90	35.74
17	221.30	219.29	221.41
18	13.48	14.27	13.21
19	19.37	19.09	18.20

Discussion

As a result of the experiment, the first identified metabolite was 3β,11α-dihydroxyandrost-5-en-17-one (2). The 1H NMR spectrum of this metabolite displayed a signal (1H, dt, J = 5 and 10 Hz) characteristic of the 11α-hydroxyl group at a chemical shift of δH 4.08 ppm. The second metabolite identified was 3β,7α-dihydroxyandrost-5-en-17-one (3), which also showed a typical 1H NMR signal (1H, bs) at δH 3.97 ppm, indicating the presence of a 7α-hydroxyl group. Isolated from Aspergillus glaucus MRC 200914, both hydroxylated forms of DHEA at the C-7α and C-11α positions were obtained. A review of the literature on steroid biotransformation using Aspergillus species revealed that hydroxylation at the C-7α position is more common than at the C-11α position. Table 2 compares the results of this experiment with those from previous studies.

Table-2. Biotransformation of DHEA with some other Aspergillus species(β=beta).

Substrate	Aspergillus	Metabolite
DHEA	Aspergillus candidus MRC 200634	3beta,17beta- Dihydroxyandroste -5-ene 6beta,17beta-Dihydroxyandroste-4-en-3-one 3beta,11α- Dihydroxyandroste -5-en-17-one 3beta,7beta-Dihydroxyandroste-5-en-17-one 3beta,7α-Dihydroxyandroste-5-en-17-one 15α, 17beta-Dihydroxyandroste-4-en-3-one (Biotransformation of some steroids by Aspergillus candidus, Kudret., (2015)).
DHEA	Aspergillus wentii MRC 200316	3beta,7beta-Dihydroxyandroste-5-en-17-one 3beta,7α-Dihydroxyandroste-5-en-17-one (Kudret, 2012).
DHEA	Aspergillus sydowii MRC 200653	6beta-Hydroxyandroste-4-en-3,17-dione 3beta,7beta-Dihydroxyandroste-5-en-17-one 3beta,7α-Dihydroxyandroste-5-en-17-one (The biotransformation of some steroids by Aspergillus sydowii MRC 200653, (Kudret 2016)).
DHEA	Aspergillus glaucus MRC 200914	3beta,11α-Dihydroxyandroste-5-en-17-one 3β,7α-Dihydroxyandroste-5-en-17-one.

Aspergillus glaucus MRC 200914 isolate hydroxylated the substrate at both C-7α and C- 11α positions, as did. Aspergillus candidus MRC 200634 isolate.

The results in Table 2 highlight the need to enhance research and experiments to discover new metabolites.

Conclusion

One of the key processes for preparing new steroid derivatives is the biotransformation of fungal steroids. These new derivatives may possess significant pharmacological activities due to their high regional and spatial selectivity. An experiment demonstrated the production of two metabolites after incubating DHEA with the isolate Aspergillus glaucus MRC 200914 for five days. The results also indicated that Aspergillus glaucus MRC 200914 is capable of hydroxylating steroids. Currently, researchers and specialists are making substantial efforts to enhance the efficiency of steroid biotransformations. This could lead to the development of useful metabolites that benefit humanity.

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Conflict of Interest

Statement Authors have no Competing Interest to Declare

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