Sakarya University Journal of Science

ISSN : 2147-835X
Publisher : Sakarya University

Vol. 28, No. 6, 1261-1266, 2024
DOI: https://doi.org/10.16984/saufenbilder.1496783

Research Article

Biotransformation of Dehydroepiandrosterone by *Penicillium olsonii* MRC500780

Ali Kuru[1*], Mena Albayaty[2]

[1] Sakarya University, Faculty of Science, Department of Chemistry, Sakarya, Türkiye, alikuru@sakarya.edu.tr
[2] Sakarya University, Institute of Natural Sciences, Sakarya, Türkiye, minazain96@gmail.com
[*]Corresponding Author

ARTICLE INFO

Keywords:
Penicillium olsonii
Steroids
Dehydroepiandrosterone
Biotransformation
Biocatalyst

Article History:
Received: 06.06.2024
Accepted: 07.12.2024
Online Available: 11.12.2024

ABSTRACT

Biotransformation is a reliable method for steroid development, with fungi serving as practical tools in this process. Fungal biocatalysts are known for their regio- and stereo-specificity, making them superior to chemical synthesis methods for steroid biotransformation. This work reported the biotransformation of dehydroepiandrosterone **1** with *Penicillium olsonii* MRC500780. A 5-day biotransformation study with the relevant microorganism resulted in 5 different products. Structure determinations using spectroscopic methods revealed that biotransformation of **1** by *Penicillium olsonii* MRC500780 afforded 5 metabolites as androst-4-ene-3,17-dione **2**, 15α-hydroxyandrost-4-ene-3,17-dione **3**, 3β,7β-dihydroxyandrost-5-en-17-one **4**, 3β,7α-dihydroxyandrost-5-en-17-one **5**, and 15α,17β-dihydroxyandrost-4-en-3-one **6**. This fungus primarily isomerized the starting material in terms of the double bond and the hydroxyl group at 3β oxidised, then hydroxylated it at the C-15α position and reduced it at the C-17 position; also, hydroxylations at the C-7α position and C-7β position were observed.

1. Introduction

It would not be easy to create a molecule with higher polarity and chemically distinct products while retaining the original carbon backbone. This problem could be solved via biotransformation, which is a structural change in a chemical substance in living cells catalyzed by microorganisms or their enzymes. [1]. Because of their extraordinary regio- and stereo-selectivity, fungus-mediated biotransformations of steroids have been extensively used for years to obtain and generate essential steroids [2]. Biotransformation converts readily available components that cannot be efficiently transformed by chemical processes into more valuable products in a microbial way, which is inexpensive. It also carries out the production of food metabolites, fine chemicals, and pharmaceuticals [3].

Natural steroid analogues could now be produced on a large scale via biotransformation. Because of their microbial conversion, these modified steroid analogues have several therapeutic benefits over their native equivalents, including greater potency, longer half-lives in the bloodstream, easier distribution, and fewer adverse effects [4].

Dehydroepiandrosterone (DHEA) is a hormone in mammals that is converted *in vivo* into androgens and estrogens [5]. Previous biotransformation investigations on DHEA by *Penicillium* species generate a diverse spectrum of metabolites due to microbial hydroxylation, hydrogenation, dehydrogenation, and, most notably, Baeyer-Villiger oxidation. [6-13].

There is no published research on DHEA biotransformation by *P. olsonii* MRC500780. The present research reported on the findings of

Cite as: A. Kuru, M. Albayaty (2024). Biotransformation of Dehydroepiandrosterone by Penicillium olsonii MRC500780, *Sakarya University Journal of Science*, 28(6), 1261-1266. https://doi.org/10.16984/saufenbilder.1496783

a 5-day DHEA biotransformation with *P. olsonii* MRC500780.

2. General Methods

2.1. Instrumentation

Varian brand, Mercury 300 model spectrometer using TMS (Merck) as an internal standard reference was used to record ^{1}H NMR spectra at 300 MHz and ^{13}C NMR spectra at 75 MHz in $CDCl_3$ (Merck). Chemical shifts are given in ppm (δ scale) and Hz for coupling constants (*J*). The Perkin Elmer brand, Spectrum Two model spectrometer was used to record infrared spectra. IA 9200 model of Electrothermal brand melting point device was used to determine the melting points. For thin layer chromatography (TLC), 0.2 mm thick plates (Merck Kieselgel 60 F_{254}) were used, and the eluent was ethyl acetate/n-hexane (1:1). The plates were immersed into a *p*-anisaldehyde (Sigma-Aldrich)/H_2SO_4 (Merck) reagent then heated to 120 ^{o}C for 3 min to see the spots.

2.2. Chemicals

Sigma-Aldrich was the source of Dehydroepiandrosterone **1** ($C_{19}H_{28}O_2$). Merck provided analytical quality solvents as well as chemicals for solid and liquid media.

2.3. Culture of the fungi

Penicillium olsonii MRC500780 was obtained from TUBITAK, Marmara Research Center. Stock cultures of the fungi were kept on potato dextrose agar (PDA) slopes at 4 ^{o}C. A liquid media was made by dissolving peptone (10 g) and glucose (30 g) in 1 L of distilled water. The medium was evenly distributed among 5 culture flasks of 250 mL capacity and autoclaved at 121 ^{o}C, 20 minutes. In a bio-safety (class II) cabinet, spores of the mould, recently collected from PDA slopes, were separated into five flasks containing sterilized medium. After cultivating each mould at 25 ^{o}C on a rotary shaker (150 rpm) for 3 days, dehydroepiandrosterone **1** (0.5 g) was dissolved in 5 ml dimethylformamide and divided equally into flasks aseptically. The fungus biotransformed the substrate in 5 flasks under identical circumstances for 5 days. For the biotransformation experiment, a control flask with non-inoculated sterile media and the relevant substrate was utilized. Each control was taken out after five days of incubation and subjected to TLC analysis. The controls showed no signs of metabolites.

2.4. Isolation of metabolites

After biotransformation, the mycelium of the mould was filtered, and the broth was eliminated using a vacuum and washed using ethyl acetate (500 mL). The broth was then extracted thrice with 1 L of ethyl acetate. After being dried over anhydrous Na_2SO_4, the organic extracts from the incubation evaporated in a vacuum, resulting in an oily substance. The steroids in the gum were separately chromatographed on silica gel 60 (Merck 107734), eluting with increasing concentrations of ethyl acetate in n-hexane. The purified substances underwent crystallisation using suitable solvents and then were identified through NMR and IR in conjunction with melting points. Metabolite quantification was expressed as conversion rate.

2.5. Biotransformation of DHEA with *Penicillium olsonii* MRC500780

After 5 days of the biotransformation of **1** with *P. olsonii* MRC500780 generated a brown gummy mixture (1017 mg), which was subsequently subjected to chromatography on silica gel, yielding the unchanged substrate (87 mg), androst-4-ene-3,17-dione **2**, 15α-hydroxyandrost-4-ene-3,17-dione **3**, 3β,7β-dihydroxyandrost-5-en-17-one **4**, 3β,7α-dihydroxyandrost-5-en-17-one **5** and 15α,17β-dihydroxyandrost-4-en-3-one **6** (Figure 1).

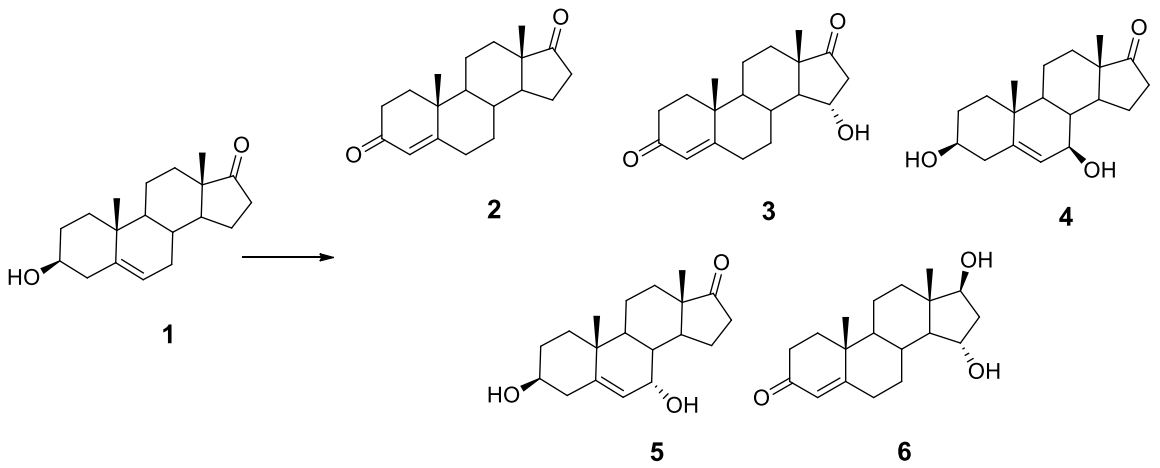

Figure 1. Biotransformation of dehydroepiandrosterone **1** by *P. olsonii* MRC500780

First product androst-4-ene-3,17–dione **2** (8%, 40 mg); m.p. 163-164 °C, lit., 158-161 °C [14].

Second product 15α-Hydroxyandrost-4-ene-3,17–dione **3** (21%, 110 mg); m.p. 185-186 °C, lit., 178-180 °C [15].

Thirth product 3β,7β–Dihydroxyandrost-5-en-17-one **4** (8%, 42 mg); m.p. 211-212 °C, lit., 207 °C [16].

Fourth product 3β,7α–Dihydroxyandrost-5-en-17-one **5** (29%, 153 mg); m.p. 201-202 °C, lit., 177 °C [16].

Product **2-6**'s ^{13}C NMR data was given in Table 1; for FT-IR data and ^{1}H NMR data, see Table 2. and Table 3.

Table 2. FT-IR data for **2-6**

Product	(Vmax/cm^{-1})
2	1735 and 1670
3	3435, 1730 and 1670
4	3250 and 1740
5	3360 and 1735
6	3390, 1655 and 1645

Table 1. ^{13}C NMR data for **1** and its metabolites

C atom	1	2	3	4	5	6
1	37.12	35.61	35.37	36.82	36.86	35.65
2	31.48	33.75	33.60	31.40	30.96	33.85
3	71.47	199.25	199.72	71.19	70.98	199.75
4	42.11	123.98	123.42	41.57	47.46	123.67
5	140.97	170.36	171.26	143.64	146.34	171.35
6	120.83	32.44	32.51	125.44	123.43	32.75
7	31.35	31.17	35.05	72.79	64.14	32.13
8	31.42	35.03	35.17	40.38	37.40	35.28
9	50.13	53.70	53.50	48.16	41.81	53.84
10	36.57	38.53	38.49	36.61	37.09	38.63
11	20.29	20.19	19.97	20.34	19.97	20.49
12	30.71	30.78	31.61	31.16	31.10	36.57
13	47.49	47.38	50.16	47.75	47.06	44.25
14	51.67	50.72	56.91	51.12	44.83	58.36
15	21.82	21.61	69.69	24.16	21.81	72.38
16	35.80	35.72	46.05	35.96	35.73	42.48
17	221.25	220.26	216.59	221.33	221.43	78.62
18	13.49	13.58	15.06	13.55	13.19	12.54
19	19.38	17.25	17.24	19.14	18.18	17.46

Table 3. ^{1}H NMR data for **2-6**

Proton	2	3	4	5	6
3α-H			1H, m, 3.53	1H, m, 3.54	
4-H	1H, s, 5.75	1H, s, 5.75			1H, s, 5.75
6-H			1H, bs, 5.30	1H, bs, 5.61	
7α-H			1H, m, 3.92		
7β-H				1H, m, 3.95	
15β-H		1H, m, 4.41			1H, m, 4.12
17α-H					1H, t, *J*=8.5Hz, 3.90
18-H	3H, s, 0.91	3H, s, 0.96	3H, s, 0.88	3H, s, 0.87	3H, s, 0.81
19-H	3H, s, 1.21	3H, s, 1.23	3H, s, 1.06	3H, s, 0.99	3H, s, 1.22

3. Result and Discussion

Incubating **1** with *P. olsonii* MRC500780 for a total of 5 days produced five products as metabolites, see (Figure 1).

androst-4-ene-3,17- dione **2** came out as the first metabolite. The multiplet peak at δ_H 3.45 ppm belonging to 3α-H was observed in the starting material **1**; however, this peak disappeared in the spectra of **2,** and the olefinic proton signal ($\Delta\delta_H$ 0.41 ppm) shifted downfield, and the signal of the methyl group at C-19 ($\Delta\delta_H$ 0.21 ppm) of **2**, indicates that the portion of **1** isomerized in terms of the double bond and hydroxyl group at 3β oxidized.

15α-hydroxyandrost-4-ene-3,17-dione **3** was found as the second metabolite. The ^{1}H NMR spectra of **1** missed the 3α-H, that a multiplet for 1 H resonance at δ_H 3.45 ppm of DHEA. A double bond signal at 0.41 ppm and the signal of the 19-methyl group ($\Delta\delta_H$ 0.23 ppm) considerably shifted downfield, and this signs the oxidation of the hydroxyl group at 3β. A multiplet peak at δH 4.41 ppm and δC 69.69 ppm confirmed that the 15α-hydroxyl group formed at **3** [17, 18]. The ^{13}C NMR spectrum of **3** had downfield shifts (Table 1.) for C-14 ($\Delta\delta_C$ 5.24 ppm) and C-16 ($\Delta\delta_C$ 10.25 ppm), further signs the 15α-hydroxyl group.

3β,7β–dihydroxyandrost–5-en-17-one **4** was defined as third product. The NMR spectra of **4** had distinctive signals; a multiplet peak for 1H at δH 3.92 ppm and a peak at δC 72.79 ppm showed the formation of a 7β-hydroxyl group [17, 18]. When looking at ^{13}C NMR spectra of **4**, C-8 shifted downfields at $\Delta\delta_C$ 8.96 ppm while C-9 exhibited an upfield shift ($\Delta\delta_C$ 1.97 ppm); thus, 7β-hydroxyl group formation was confirmed.

3β,7α-dihydroxyandrost-5-en-17-one **5** was identified as the fourth metabolite. The NMR spectra of **5** revealed different features. [17, 18]; 7α-hydroxyl group formation was verified through a multiplet peak for 1H at δ_H 3.95 ppm and a peak at δ_C 64.14 ppm. When the ^{13}C NMR spectra of **5** was analyzed, it was seen that C-8 shifted downfield, and a peak was observed at $\Delta\delta_C$ 5.98 ppm; thus, 7α-hydroxyl group formation was approved.

15α,17β-dihydroxyandrost-4-en-3-one **6** was recognized as the fifth metabolite. When the ^{1}H NMR spectra were examined, it was seen that the multiplet peak at δ_H 3.45, ppm belonging to 3α-H of **1**, disappeared at **6**. A signal at $\Delta\delta_H$ 0.41 ppm belonging to olefinic proton shifted downfield. The signal of the methyl group at C-19 ($\Delta\delta_H$ 0.22 ppm) belonging **1**, designating the isomerisation in terms of the double bond and oxidation of hydroxyl group at 3β. Compound **6**'s NMR spectra revealed two distinct signals: a triplet signal for 1H at δ_H 3.90 ppm (J = 8.5 Hz) and a multiplet signal for 1H at δ_H 4.12 ppm showing respectively the presence of 17β- and 15α-hydroxyl groups [14]. The ^{13}C NMR spectra of **6** contained two novel signals: a signal at δ_C 72.38 ppm and another signal at δ_C 78.62 ppm, further showing respectively the presence of 15α- and 17β-hydroxyl groups [15].

4. Conclusion

Penicillium olsonii MRC500780 primarily oxidized the hydroxyl group at the 3β position, besides shifting the double bound to 4-en. The majority of the starting material is hydroxylated at the position of C-15α, with small reduction at C-17, besides some of **1** hydroxylated at C-7α position and C-7β position by *P. olsonii* MRC500780.

Penicillium species have previously converted the hydroxyl group at 3β of the starting substance to a carbonyl group while shifting the double bond at 5-en, followed by hydroxylation at C-15α [6-13]. *P. olsonii* MRC500780 was the first to carry out C-7α and C-7β hydroxylations, as well as a C-17 reduction on **1**. Our research on the biotransformation of additional steroids by *Penicillium olsonii* MRC500780 and other fungi is ongoing.

Article Information Form

Acknowledgements
The authors would like to thank Prof. Dr. Kudret Yıldırım for his great contributions and Yavuz Derin for running ^{1}H NMR and ^{13}C NMR spectra.

Funding
This work was financially supported by the Sakarya University Scientific Research Foundation (Project no. 2022-7-24-160)

Authors' Contribution
A. Kuru: Supervision, project administration and management, data analysis, writing.
M. Albayaty: Performing the experiments and collecting the data.

The Declaration of Conflict of Interest/ Common Interest
No conflict of interest or common interest has been declared by the authors.

The Declaration of Ethics Committee Approval
This study does not require permission from the ethics committee or any special permission.

The Declaration of Research and Publication Ethics
The authors of the paper declare that they comply with the scientific, ethical and quotation rules of SAUJS in all processes of the paper and that they do not make any falsification of the data collected. In addition, they declare that Sakarya University Journal of Science and its editorial board have no responsibility for any ethical violations that may be encountered and that this study has not been evaluated in any academic publication environment other than Sakarya University Journal of Science.

References

[1] M. S. Smitha, S. Singh, R. Singh, "Microbial biotransformation: A process for chemical alterations", Journal of Bacteriology & Mycology: Open Access, vol. 4, no.2, p. 85, 2017.

[2] N. Nassiri-Koopaei, M. A. Faramarzi, "Recent developments in the fungal transformation of steroids", Biocatalysis and Biotransformation, vol. 33, no.1, pp. 1-28, 2015.

[3] V. Dave, P. Khirwadkar, K. Dashora, "A review on biotransformation", Indian Journal of Research in Pharmacy and Biotechnology, vol. 2, no. 2, p. 1136, 2014.

[4] S.S. Mohamed, A. A. El-Hadi, K. M. Abo-Zied, "Biotransformation of prednisolone to hydroxy derivatives by Penicillium aurantiacum", Biocatalysis and Biotransformation, vol. 35, no.3, pp. 215-222, 2017.

[5] M. Panjari, S. R. Davis, "DHEA therapy for women: Effect on sexual function and wellbeing", Human reproduction update, vol. 13, no. 3, pp. 239-248, 2007.

[6] L.H. Huang, J. Li, G. Xu, X. H. Zhang, Y. G. Wang, Y. L. Yin, H. M. Liu, "Biotransformation of dehydroepiandrosterone (DHEA) with Penicillium griseopurpureum Smith and Penicillium glabrum (Wehmer) Westling", Steroids, vol. 75, no.13, pp. 1039-1046, 2010.

[7] A. Swizdor, "Baeyer-villager oxidation of some C19 steroids by penicillium lanosocoeruleum", Molecules, vol. 18, no. 11, pp. 13812–13822, 2013.

[8] T. Kołek, A. Szpineter, A. Świzdor, "Baeyer-Villiger oxidation of DHEA, pregnenolone, and androstenedione by Penicillium lilacinum AM111", Steroids, vol. 73, no. 14, pp. 1441–1445, 2008.

[9] A. Panek, P. Łyczko, A. Świzdor, "Microbial modifications of androstane and androstene steroids by Penicillium vinaceum", Molecules, vol. 25, no. 18, p. 4226, 2020.

[10] E. Kozłowska, M. Urbaniak, A. Kancelista, M. Dymarska, E. Kostrzewa-Susłow, Ł. Stępień, T. Janeczko, "Biotransformation of dehydroepiandrosterone (DHEA) by environmental strains of filamentous fungi", RSC advances, vol. 7, no. 50, pp. 31493-31501, 2017.

[11] H. M. Liu, H. Li, L. Shan, J. Wu, "Synthesis of steroidal lactone by penicillium citreo-viride", Steroids, vol. 71, no. 11, pp. 931-934, 2006.

[12] B. Yang, Y. Wang, X. Chen, J. Feng, Q. Wu, D. Zhu, Y. Ma, "Biotransformations of steroids to testololactone by a multifunctional strain Penicillium simplicissimum WY134-2", Tetrahedron, vol. 70, no. 1, pp. 41-46, 2014.

[13] T. Kołek, A. Szpineter, A. Świzdor, "Studies on Baeyer–Villiger oxidation of steroids: DHEA and pregnenolone d-lactonization pathways in Penicillium camemberti AM83", Steroids, vol. 74, no. 10, pp. 859-862, 2009.

[14] T. Iida, K. Omura, R. Sakiyama, M. Kodomari, "Regioselective dehydrogenation of 3-keto-steroids to form conjugated enones using o-iodoxybenzoic acid and trifluoroacetic acid catalysis", Chemistry and Physics of Lipids, vol. 178, pp. 45-51, 2014.

[15] P. C. Peart, K. P. McCook, F. A. Russell, W. F. Reynolds, P. B. Reese, "Hydroxylation of steroids by Fusarium oxysporum, Exophiala jeanselmei and Ceratocystis paradoxa", Steroids, vol. 76, no.12, pp. 1317-1330, 2011.

[16] C. S. Bensasson, J. R. Hanson, Y. Le Huerou, "The microbiological hydroxylation of 3α, 5-cycloandrostanes by Cephalosporium aphidicola", Phytochemistry, vol. 52, no. 7, pp. 1279-1282, 1999.

[17] D. N. Kirk, H. C. Toms, C. Douglas, K. A. White, K. E. Smith, S. Latif, R.W.P Hubbard, "A survey of the high-field ^{1}H NMR spectra of the steroid hormones, their hydroxylated derivatives, and related compounds", Journal of the Chemical Society, Perkin Transactions, vol. 2, pp. 1567-1594, 1990.

[18] J. W. Blunt, J. B. Stothers, "^{13}C NMR spectra of steroids a survey and commentary", Organic Magnetic Resonance, vol. 9, pp. 439-464, 1977.