Biocatalysis and Biotransformation

ISSN: 1024-2422 (Print) 1029-2446 (Online) Journal homepage: https://www.tandfonline.com/loi/ibab20

Biotransformation of testosterone by *Cladosporium sphaerospermum*

Kudret Yildirim, Ali Kuru & Şengül Yılmaz

To cite this article: Kudret Yildirim, Ali Kuru & Şengül Yılmaz (2019): Biotransformation of testosterone by *Cladosporium sphaerospermum*, Biocatalysis and Biotransformation, DOI: 10.1080/10242422.2019.1583747

To link to this article: https://doi.org/10.1080/10242422.2019.1583747

Published online: 15 Mar 2019.

BIOCATALYSIS AND BIOTRANSFORMATION
https://doi.org/10.1080/10242422.2019.1583747

Taylor & Francis
Taylor & Francis Group

RESEARCH ARTICLE

Biotransformation of testosterone by *Cladosporium sphaerospermum*

Kudret Yildirim, Ali Kuru and Şengül Yılmaz

Department of Chemistry, Faculty of Arts and Sciences, Sakarya University, Sakarya, Turkey

ABSTRACT

Incubation of testosterone **1** with *Cladosporium sphaerospermum* MRC 70266 afforded six metabolites and two of these metabolites, 6β,16β,17β-trihydroxyandrost-4-en-3-one **6** and 6β,12β,17β-trihydroxyandrost-4-en-3-one **7,** were determined as new compounds. The fungus mainly hydroxylated testosterone **1** at C-6β, accompanied by some minor hydroxylations at C-7β, C-12β, C-15α and C-16β. A minor oxidation at C-17 and a minor 5α-reduction were also observed.

ARTICLE HISTORY
Received 27 December 2018
Accepted 8 February 2019

KEYWORDS
Testosterone; biotransformation; *Cladosporium sphaerospermum*

Introduction

Microbial steroid biotransformations have found worldwide application for the preparation of more valuable steroidal drugs and hormones due to their high regio- and stereoselectivities. There are still enormous efforts to perform more effective microbial steroid biotransformations and to detect new useful microorganisms and reactions (Donova and Egorova 2012; Nassiri-Koopaei and Faramarzi 2015).

Cladosporium, belonging to the Cladosporiaceae family of the Ascomycota, is a large genus (Sandoval-Denis et al. 2016). *Cladosporium* species are cosmopolitan in distribution and usually isolated from air, food, plants, paint and textiles (Bensch et al. 2012; Sandoval-Denis et al. 2016). These species are common endophytes, plant pathogens and even hyperparasites of other fungi (Sandoval-Denis et al. 2016). Some *Cladosporium* species are also considered pathogenic to humans and animals (Sandoval-Denis et al. 2015).

Cladosporium sphaerospermum is a halotelorant or osmotelorant fungus widely distributed around the world (Zalar et al. 2007). It is frequently isolated from indoor and outdoor locations (Aihara et al. 2001; Park et al. 2004), humans (Yano et al. 2003) and plants (Pereira et al. 2002).

As far as steroid biotransformations by *C. sphaerospermum* are concerned, we have not found any literature work on steroids. In this work, testosterone **1** was incubated with *C. sphaerospermum* MRC 70266 for 5 d in order to investigate its metabolism.

Materials and methods

Instrumental methods

^{1}H NMR spectra were recorded in deuteriochloroform with tetramethylsilane as an internal standard reference at 300 MHz with a Varian Mercury 300 spectrometer (Palo Alto, CA). ^{13}C NMR spectra were recorded in deuteriochloroform at 75 MHz with a Varian Mercury 300 spectrometer. Chemical shifts are given in ppm (δ scale), and coupling constants (J) are given in Hz. Infrared spectra were recorded using a Perkin Elmer Spectrum Two spectrometer (Whaltam, MA). Elemental analysis was performed using a Thermo Finnigan Flash EA 1112 elemental analyser (Bremen, Germany). Melting points were determined by an Electrothermal IA 9200 melting point apparatus (Staffordshire, UK) and are uncorrected. Thin layer chromatography (TLC) was carried out with 0.2 mm thick Merck Kieselgel 60 F_{254} TLC plates (Istanbul, Turkey) using ethyl acetate/*n*-hexane (1:1) as eluent. TLC plates were dipped into an anisaldehyde/H_2SO_4 reagent and heated to 120 °C for 3 min in order to visualize the spots.

Chemicals

Testosterone **1** was purchased from Sigma-Aldrich (Istanbul, Turkey). Solvents were of analytical grade and were purchased from Merck (Istanbul, Turkey). Potato dextrose agar (PDA) and agar for PDA slopes

CONTACT Kudret Yildirim kudrety@sakarya.edu.tr Department of Chemistry, Faculty of Arts and Sciences, Sakarya University, Sakarya 54187, Turkey

Supplemental data for this article can be accessed here.

and ingredients for liquid medium were also purchased from Merck (Istanbul, Turkey).

Micro-organism and fermentation conditions

Cladosporium sphaerospermum MRC 70266 was obtained from TUBITAK, Marmara Research Center, Food Science and Technology Research Institute, Culture Collection Unit, Kocaeli, Turkey. Stock cultures were maintained at on PDA slopes at 4 °C.

The liquid medium for the *C. sphaerospermum* MRC 70266 was prepared by mixing glucose (20 g), peptone (5 g) and yeast extract (5 g) in 1 L of distilled water (Shebany 2012). The medium was evenly distributed among 10 culture flasks of 250 mL capacity (100 mL in each) and autoclaved for 20 min at 121 °C. Spores freshly obtained from PDA slopes were transferred aseptically into each flask containing sterile medium in a biological safety cabinet. After cultivation at 28 °C for 3 d on a rotary shaker (160 rpm), testosterone **1** (1 g) dissolved in 10 ml of DMF was evenly distributed aseptically among the flasks. The biotransformation of the substrate was carried out in 10 flasks for 5 d under the same conditions. Biotransformation experiment was run with a control flask containing non-inoculated sterile medium and the substrate. After 5 d of incubation, the control was harvested and analysed by TLC. No metabolites were detected in the control.

Isolation and identification of metabolites

After incubation, the fungal mycelium was separated from the broth by filtration under vacuum, and the mycelium was rinsed with ethyl acetate (500 ml). The broth was then extracted three times with each 1 L of ethyl acetate. The organic extract was dried over anhydrous sodium sulphate, and the solvent evaporated *in vacuo* to give a brown gum (1759 mg). The steroids in brown gum were chromatographed on silica gel 60 (Merck 107734), eluting with increasing concentrations of ethyl acetate in *n*-hexane. Steroid mixtures from the column were then separated by column chromatography on aluminium oxide 90 active neutral (activity stage I, Merck 101077), using smaller columns for 2–3 h. The purified steroids were crystallized in appropriate solvents and identified by using melting points and a combination of NMR, IR and elemental analysis.

Biotransformation of testosterone 1 by C. sphaerospermum MRC 70266

Incubation of testosterone **1** with *C. sphaerospermum* MRC 70266 for 5 d afforded 6 metabolites **2**–**7** (Figure 1). Elution with ethyl acetate in *n*-hexane (2:3) afforded the unreacted substrate (97 mg), which was identified by comparison of its ^{1}H and ^{13}C NMR spectra with those of an authentic sample.

Elution with ethyl acetate in *n*-hexane (1:1) afforded 6β-hydroxyandrost-4-en-3,17-dione **2** (84 mg, 8%); crystallized from acetone as prisms; mp 189–190 °C, lit (Hanson et al. 1996); mp 190–191 °C; IR ν_{max} 3415, 1740, 1670 cm^{-1}; EA: calculated for $C_{19}H_{26}O_3$: C 75.46, H 8.67; found: C 75.32, H 8.53%; ^{1}H NMR ($CDCl_3$): δ_H 0.91 (3H, s, 18-H), 1.38 (3H, s, 19-H), 4.36 (1H, brs, 6α-H), 5.80 (1H, s, 4-H); ^{13}C NMR ($CDCl_3$): see Table 1.

Elution with ethyl acetate in *n*-hexane (3:2) afforded 6β,17β-dihydroxyandrost-4-en-3-one **3** (422 mg, 40%); crystallized from ethyl acetate as prisms; mp 212–213

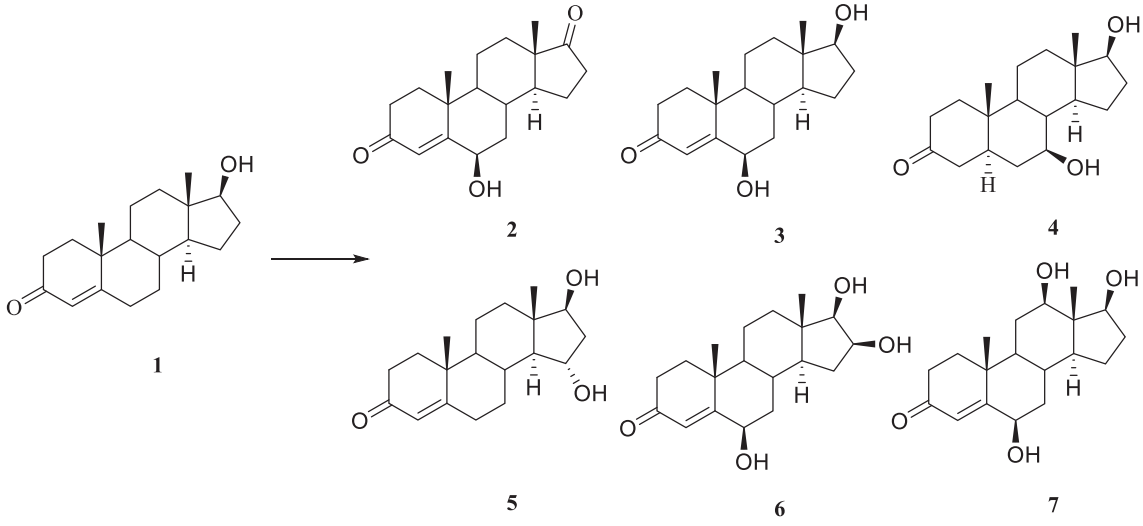

Figure 1. Biotransformation of testosterone **1** by *C. sphaerospermum* MRC 70266.

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

C atom	1	2	3	4	5	6	7
1	35.48	36.97	36.37	36.93	35.68	37.04	35.02
2	33.74	34.09	34.19	34.24	33.89	34.19	34.13
3	199.64	200.33	200.41	214.07	199.61	200.47	200.26
4	123.87	126.34	126..32	34.61	123.76	126.38	126.53
5	171.52	168.06	168.32	39.91	171.07	168.14	167.67
6	32.63	72.58	72.96	29.96	32.72	72.90	72.81
7	31.33	37.17	37.09	69.63	32.14	38.04	37.43
8	35.58	29.34	29.75	37.81	35.29	29.17	28.84
9	53.69	53.57	53.64	59.24	53.81	53.81	52.46
10	38.45	38.00	38.01	36.50	38.63	38.15	37.95
11	20.10	20.19	20.56	20.26	20.50	20.27	29.33
12	36.21	31.19	38.01	42.25	36.56	36.98	78.99
13	42.61	47.57	42.88	43.29	44.31	42.43	46.98
14	50.24	50.81	50.44	51.29	58.43	46.94	48.51
15	23.14	21.64	23.25	23.04	72.46	34.91	23.07
16	29.51	35.71	30.42	29.44	42.57	69.85	30.01
17	81.25	220.62	81.64	81.12	78.70	80.62	81.87
18	10.93	13.71	11.07	10.96	12.56	11.88	6.02
19	17.17	19.46	19.51	22.98	17.48	19.52	19.44

°C, lit (Hanson et al. 1996); mp 215–220 °C; IR ν_{max} 3405, 1650, 1620 cm^{-1}; EA: calculated for $C_{19}H_{28}O_3$: C 74.96, H 9.27; found: C 74.82, H 9.13%; ^1H NMR ($CDCl_3$): δ_H 0.82 (3H, s, 18-H), 1.38 (3H, s, 19-H), 3.65 (1H, t, J = 8.5 Hz, 17α-H), 4.36 (1H, brs, 6α-H), 5.80 (1H, s, 4-H); ^{13}C NMR ($CDCl_3$): see Table 1.

Elution with ethyl acetate in *n*-hexane (7:3) afforded 7β,17β-dihydroxyandrostan-3-one **4** (75 mg, 7%); crystallized from ethyl acetate as needles; mp 202–203 °C lit (Farooq and Tahara, 2000); mp 196–197 °C; IR ν_{max} 3325, 1710 cm^{-1}; EA: calculated for $C_{19}H_{30}O_3$: C 74.47, H 9.87; found: C 74.33, H 9.70%; ^1H NMR ($CDCl_3$): δ_H 0.74 (3H, s, 19-H), 0.83 (3H, s, 18-H), 3.65 (2H, m, 7α-H and 17α-H); ^{13}C NMR ($CDCl_3$): see Table 1.

Elution with ethyl acetate in *n*-hexane (9:1) afforded 15α,17β-dihydroxyandrost-4-en-3-one **5** (53 mg, 5%); crystallized from acetone as needles; mp 96–97 °C, lit (Peart et al. 2011); mp 93–94 °C; IR ν_{max} 3400, 1660, 1650 cm^{-1}; EA: calculated for $C_{19}H_{28}O_3$: C 74.96, H 9.27; found: C 74.85, H 9.17%; ^1H NMR ($CDCl_3$): δ_H 0.80 (3H, s, 18-H), 1.20 (3H, s, 19-H), 3.89 (1H, t, J = 8.5 Hz, 17α-H), 4.11 (1H, m, 15β-H), 5.73 (1H, s, 4-H); ^{13}C NMR ($CDCl_3$): see Table 1.

Elution with pure ethyl acetate afforded 6β,16β,17β-trihydroxyandrost-4-en-3-one **6** (34 mg, 3%); crystallized from ethyl acetate as cubes; mp 169–170 °C; IR ν_{max} 3395, 1660 cm^{-1}; EA: calculated for $C_{19}H_{28}O_4$: C 71.22, H 8.81; found: C 71.15, H 8.70%; ^1H NMR ($CDCl_3$): δ_H 0.88 (3H, s, 18-H), 1.39 (3H, s, 19-H), 3.38 (1H, d, J = 7.3 Hz, 17α-H), 4.16 (1H, m, 16α-H), 4.34 (1H, brs, 6α-H), 5.81 (1H, s, 4-H); ^{13}C NMR ($CDCl_3$): see Table 1.

Further elution with pure ethyl acetate afforded 6β,12β,17β-trihydroxyandrost-4-en-3-one **7** (23 mg, 2%); crystallized from ethyl acetate as needles; mp 200–201 °C; IR ν_{max} 3445, 1680 cm^{-1}; EA: calculated for $C_{19}H_{28}O_4$: C 71.22, H 8.81; found: C 71.10, H 8.73%; ^1H NMR ($CDCl_3$): δ_H 0.87 (3H, s, 18-H), 1.39 (3H, s, 19-H), 3.50 (1H, dd, J = 5.0, 11.0Hz, 12α-H), 3.89 (1H, t, J = 8.5 Hz, 17α-H), 4.35 (1H, brs, 6α-H), 5.81 (1H, s, 4-H); ^{13}C NMR ($CDCl_3$): see Table 1.

Results

The first metabolite was identified as 6β-hydroxyandrost-4-en-3,17-dione **2**. NMR spectra of **2** had new resonances at δ_H 4.36 ppm (1H, brs) and δ_C 72.58 ppm (see Supplemental data), which were typical for the presence of a 6β-hydroxyl group (Hanson et al. 1996). The ^{13}C NMR spectrum of **2** lacked the C-17 resonance of **1** at δ_C 81.25 ppm and had a new carbon atom resonance at δ_C 220.62 ppm, suggesting that an oxidation had taken place at C-17.

The second metabolite was identified as 6β,17β-dihydroxyandrost-4-en-3-one **3**. The ^1H NMR spectrum of **3** showed characteristic resonances at δ_H 3.65 ppm (1H, t, J = 8.5 Hz) and δ_H 4.36 ppm (1H, brs), indicating the presence of 17β- and 6β-hydroxyl groups, respectively (Hanson et al. 1996). The ^{13}C NMR spectrum of **3** had resonances at δ_C 72.96 and δ_C 81.64 ppm, which were in agreement with the presence of 7β- and 17β-hydroxyl groups, respectively.

The third metabolite was identified as 7β,17β-dihydroxyandrostan-3-one **4**. The ^1H NMR spectrum of **4** lacked the 4-H resonance of **1** at δ_H 5.74 ppm (1H, s), suggesting the hydrogenation of the double bond in ring A. The ^{13}C NMR spectrum of **4** had two resonances at δ_C 69.93 ppm and δ_C 81.12 ppm, which were typical for the presence of 7β- and 17β-hydroxyl groups, respectively (Farooq and Tahara 2000). The ^1H NMR spectrum of **4** showed two overlapping resonances at δ_H 3.65 ppm (2H, m), which were in agreement with the presence of 7β- and 17β-hydroxyl groups.

The fourth metabolite was identified as 15α,17β-dihydroxyandrost-4-en-3-one **5**. The ^{13}C NMR spectrum of **5** showed two characteristic resonances at δ_H 3.89 ppm (1H, t, J = 8.5 Hz) and δ_H 4.11 ppm (1H, m), indicating the presence of 17β- and 15α-hydroxyl groups, respectively (Peart et al. 2011). The ^{13}C NMR spectrum of **5** showed two resonances at δ_C 72.46 and δ_C 78.70 ppm, further indicating the presence of 15α- and 17β-hydroxyl groups, respectively.

The fifth metabolite was identified as 6β,16β,17β-trihydroxyandrost-4-en-3-one **6**. The ^{13}C NMR spectrum of **6** had two resonances at δ_C 72.90 ppm and δ_C 80.62 ppm, which were characteristic (Hanson et al. 1996) for the presence of 6β- and 17β-hydroxyl

groups, respectively. The NMR spectra of **6** showed new resonances at δ_H 4.16 ppm (1H, m) and δ_C 69.85 ppm, indicating the presence of another hydroxyl group. The characteristic triplet of 17α-H (Bridgeman et al. 1970) was observed as a doublet due to splitting by a proton at C-16. The ^{13}C NMR spectrum of **6** showed a downfield shift for C-15 ($\Delta\delta_C$ 11.77 ppm) whereas it showed a γ-gauche upfield shift for C-14 ($\Delta\delta_C$ 3.30 ppm), further indicating the presence of a hydroxyl group at C-16. According to the literature, the change in the characteristic multiplicity of 17α-H resonance suggested the presence of a 16β-hydroxyl group (Świzdor et al. 2017). The ^{13}C NMR spectrum of the metabolite exhibited resonances for 19 carbons while its DEPT spectra revealed two methyl, six methylene, seven methine and four quaternary carbons, which were in accordance with the suggested structure of the triol. 6β,16β,17β-Trihydroxyandrost-4-en-3-one **6** was determined as a new metabolite.

The sixth metabolite was identified as 6β,12β,17β-trihydroxyandrost-4-en-3-one **7**. The ^{13}C NMR spectrum of **7** showed characteristic resonances at δ_C 72.81 ppm and δ_C 81.87 ppm, suggesting the presence of 6β- and 17β-hydroxyl (Hanson et al. 1996) groups, respectively. The ^{13}C NMR spectrum of **7** also showed another characteristic resonance at δ_C 78.99 ppm, indicating the presence a 12β-hydroxyl group (Hunter et al. 2008). The ^{13}C NMR spectrum of **7** showed a downfield shift for C-11 ($\Delta\delta_C$ 9.23 ppm) whereas it showed a γ-gauche upfield shift for C-14 ($\Delta\delta_C$ 1.73 ppm), further indicating the presence of a 12β-hydroxyl group. The presence of two methyl, six methylene, seven methine and four quaternary carbons in the DEPT spectra of **7** were in agreement with the proposed structure of the triol. 6β,12β,17β-Trihydroxyandrost-4-en-3-one **7** was determined as a new metabolite.

Discussion

As can be seen from Table 2, *C. sphaerospermum* MRC 70266 hydroxylated testosterone **1** predominantly at C-6β and some minor hydroxylations took place at C-7β, C-12β, C-15α and C-16β. In addition to this, a minor oxidation at C-17 and a minor 5α-reduction were also observed. In a recent work, however, *Cladosporium cladosporioides* MRC 70282, another *Cladosporium* isolate, metabolised **1** in a very different way. This fungus only hydroxylated **1** at C-16β and most of this new hydroxyl group was then oxidised to a carbonyl group. A minor independent oxidation at C-17 and a minor epimerisation at C-17 were also observed (Yildirim et al. 2018a).

Table 2. Metabolite yields following chromatography.

Substrate	Metabolite	% Yield
Testosterone **1**		
	6β-Hydroxyandrost-4-en-3,17-dione **2**	8
	6β,17β-Dihydroxyandrost-4-en-3-one **3**	40
	7β,17β-Dihydroxyandrostan-3-one **4**	7
	15α,17β-Dihydroxyandrost-4-en-3-one **5**	5
	6β,16β,17β-Trihydroxyandrost-4-en-3-one **6**	3
	6β,12β,17β-Trihydroxyandrost-4-en-3-one **7**	2

Reports of fungal 5α-reduction, oxidation at C-17 and hydroxylation of **1** at C-6β, C-15α and C-16β are very common (Mahato and Garai 1997; Fernandes et al. 2003; Bhatti and Khera 2012; Donova and Egorova 2012; Nassiri-Koopaei and Faramarzi 2015). However, fungal hydroxylations of **1** at C-7β and C-12β are very rare (Tweit et al. 1962; Smith et al. 1990; Farooq and Tahara 2000; Hunter et al. 2008; Yildirim et al. 2018b). In a previous work, for example, *Botryosphaerica obtusa* hydroxylated **1** at C-7β, C-11α, C-12β and C-15α (Smith et al. 1990). In another work, *Botrytis cinerea* hydroxylated **1** at C-7β and reduced its C4–C5 double bond from the α-face (Farooq and Tahara, 2000). *Rhizomucor tauricus* (Hunter et al. 2008) hydroxylated **1** at C-6β and C-12β whilst a *Penicillium* isolate (Tweit et al. 1962) hydroxylated **1** at C-1α, C-6β, C-12β and C-15β. In a recent work, *Ulocladium chartarum* MRC 72584 hydroxylated **1** at C-6β, C-7β, C-12β and C-14α, accompanied by a 5α-reduction and oxidations at C-6 and C-17 (Yildirim et al. 2018b). However, in the present work, as stated above, *C. sphaerospermum* MRC 70266 hydroxylated **1** at C-6β, C-7β, C-12β, C-15α and C-16β, accompanied by a 5α-reduction and an oxidation at C-17.

In short, we were able to see how testosterone **1** was metabolised by *C. sphaerospermum* MRC 70266. It was shown that the fungus mainly hydroxylated **1** at C-6β, accompanied by a minor oxidation at C-17, a minor 5α-reduction and some minor hydroxylations at C-7β, C-12β, C-15α and C-16β. In addition to this, two of the metabolites, 6β,16β,17β-trihydroxyandrost-4-en-3-one **6** and 6β,12β,17β-trihydroxyandrost-4-en-3-one **7**, were determined as new compounds. Our work on biotransformations of some other steroids by *C. sphaerospermum* MRC 70266 and some other fungi is in progress.

Acknowledgements

The authors are grateful to Raşit Fikret Yılmaz and Fatih Sonmez for running the NMR spectra.

Disclosure statement

The authors report no declaration of interest. The authors alone are responsible for the content and writing of the paper.

ORCID

Kudret Yildirim http://orcid.org/0000-0002-7889-5903
Ali Kuru http://orcid.org/0000-0002-8300-1572
Şengül Yılmaz http://orcid.org/0000-0001-5374-7448

References

Aihara M, Tanaka T, Takatori K. 2001. *Cladosporium* as the main fungal contaminant of locations in dwelling environments. Biocontrol Sci. 6:49–52.

Bensch K, Braun U, Groenewald JZ, Crous PW. 2012. The genus *Cladosporium*. Stud Mycol. 72:1–401.

Bhatti HN, Khera RA. 2012. Biological transformations of steroidal compounds: a review. Steroids. 77:1267–1290.

Bridgeman JE, Cherry PC, Clegg AS, Evans JM, Jones ERH, Kasal A, Kumar V, Meakins GD, Morisawa Y, Richards EE, et al. 1970. Microbiological hydroxylation of steroids. Part I. Proton magnetic resonance spectra of ketones, alcohols, and acetates in the androstane, pregnane, and oestrane series. *J Chem Soc C*. 250–257.

Donova MV, Egorova OV. 2012. Microbial steroid transformations: current state and prospects. Appl Microbiol Biotechnol. 94:1423–1447.

Farooq A, Tahara S. 2000. Biotransformation of testosterone and pregnenolone catalyzed by the fungus *Botrytis cinerea*. J Nat Prod. 63:489–491.

Fernandes P, Cruz A, Angelova B, Pinheiro HM, Cabral JMS. 2003. Microbial conversion of steroid compounds: recent developments. Enzyme Microbial Technol. 32:688–705.

Hanson JR, Nasir H, Parvez A. 1996. The hydroxylation of testosterone and some relatives by *Cephalosporium aphidicola*. Phytochemistry. 42:411–415.

Hunter AC, Mills PW, Dedi C, Dodd HT. 2008. Predominant allylic hydroxylation at carbons 6 and 7 of 4 and 5-ene functionalized steroids by the thermophilic fungus *Rhizomucor tauricus* IMI23312. J Steroid Biochem Mol Biol. 108:155–163.

Mahato SB, Garai S. 1997. Advances in microbial steroid biotransformation. Steroids. 62:332–345.

Nassiri-Koopaei N, Faramarzi MA. 2015. Recent developments in the fungal transformation of steroids. Biocatal Biotransform. 33:1–28.

Park HG, Managbanag JR, Stamenova EK, Jong SC. 2004. Comparative analysis of common indoor *Cladosporium* species based on molecular data and conidial characters. Mycotaxon. 89:441–451.

Peart PC, McCook KP, Russell FA, Reynolds WF, Reese PB. 2011. Hydroxylation of steroids by *Fusarium oxysporum*, *Exophiala jeanselmei* and *Ceratocystis paradoxa*. Steroids. 76:1317–1330.

Pereira PT, De Carvalho MM, Girio FM, Roseiro JC, Amaral CMT. 2002. Diversity of microfungi in the phylloplane of plants growing in a Mediterranean ecosystem. J Basic Microbiol. 42:396–407.

Sandoval-Denis M, Sutton DA, Martin-Vicente A, Cano-Lira JF, Wiederhold N, Guarro J, Gene J. 2015. *Cladosporium* species recovered from clinical samples in the United States. J Clin Microbiol. 53:2990–3000.

Sandoval-Denis M, Gené J, Sutton DA, Wiederhold NP, Cano-Lira JF, Guarro J. 2016. New species of *Cladosporium* associated with human and animal infections. Persoonia. 36:281–298.

Shebany YM. 2012. Antimicrobial activity of endophytic fungi isolated from *Avicennia marina* plant, Red Sea. Egypt J Microbiol. 47:141–152.

Smith KE, Latif S, Kirk DN. 1990. Microbial transformations of steroids-VI. Transformation of testosterone and androstenedione by *Botryosphaerica obtusa*. J Steroid Biochem. 35:115–120.

Świzdor A, Panek A, Milecka-Tronina N. 2017. Hydroxylative activity of *Aspergillus niger* towards androst-4-ene and androst-5-ene steroids. Steroids. 126:101–106.

Tweit RC, Dodson RM, Muir RD. 1962. Microbiological transformations. XII. The substrate specificity of hydroxylations by a *Penicillium* sp., A.T.C.C. 12,556. J Org Chem. 27: 3654–3658.

Yano S, Koyabashi K, Kato K. 2003. Intrabronchial lesion due to *Cladosporium sphaerospermum* in a healthy, non-asthmatic woman. Mycoses. 46:348–350.

Yildirim K, Kuru A, Yılmaz RF. 2018a. Microbial transformation of some steroids by *Cladosporium cladosporioides* MRC 70282. *J Chem Res*. 42:408–411.

Yildirim K, Kuru A, Yılmaz Ş. 2018b. Biotransformation of testosterone by *Ulocladium chartarum* MRC 72584. *J Chem Res*. 42:444–446.

Zalar P, de Hoog GS, Schroers H-J, Crous PW, Groenewald JZ, Gunde-Cimerman N. 2007. Phylogeny and ecology of the ubiquitous saprobe *Cladosporium shaerospermum*, with descriptions of seven new species from hypersaline environments. Stud Mycol. 58:157–183.