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Hydroxylation of steroid compounds by *Gelasinospora retispora*

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ABSTRACT

Biotransformation of androst-4-ene-3,17-dione (**1**), 3β-hydroxypregnan-5-en-20-one (pregnenolone, **2**), 3β-hydroxyandrost-5-en-17-one (DHEA, **3**) and estradiol (**4**) was investigated with fungus of *Gelasinospora retispora*. Biotransformation of **1** gave 11α-hydroxyandrost-4-ene-3,17-dione (**5**) in good yield. In the case of compound **2**, three compounds, DHEA (**3**), 3β,17β-dihydroxyandrost-5-ene (**6**) and 3β,15β-dihydroxyandrost-5-en-17-one (**7**) were obtained. Moreover, DHEA (**3**) was converted to 3β,7α-dihydroxyandrost-5-en-17-one (**8**) and 3β,11α-dihydroxyandrost-5-ene-7,17-dione (**9**). And it was found that biotransformation of **4** affords 6β-hydroxyestradiol (**10**).

1. Introduction

From the viewpoint of green chemistry, biotransformations constitute an important method in organic chemistry. This technique has been widely used for the transformation and synthesis of steroids [1]. Since 1950s the biosynthesis and conversion of steroids by microorganisms have been studied [2,3]. These studies showed that they can perform the oxidation–reduction reaction and decomposition reaction of steroids. In particular, hydroxylations are one of the key reactions of steroid compounds in microorganisms. They have been reported as more important reactions since discovery of the therapeutic value of cortisone [4], and 11α-hydroxylation of progesterone by *Rhizopus arrhizus*. Many examples of 11α-hydroxylation for biotransformation have been described in the literature [3]. One of the principal biotransformations is 11α-hydroxyprogesterone by *R. nigricans* [5]. Moreover, the possibility for the introduction of one or more hydroxyl group at different positions of steroid nucleus was studied [6–14]. On the other hand, hydroxylation at C-15 position by various microorganisms has been reported [13–15]: for example, Gubler et al. and Al-Aboudi et al. reported that C-15α position was hydroxylated by the genus *Fusarium* and C-15β position by the genus *Aspergillus*. These developments were widely applicable for steroidal industrial fields [16].

About 1500 species, containing *Streptomyces*, *Aspergillus* and *Penicillium*, of fungi have been studied about conversion of steroid. Moreover, it is necessary to inquire about much more microorganisms to develop the inexpensive industrial production of steroidal drugs. However, there is only very little information on the biotransformation of steroid using the genus *Gelasinospora* [17]. Here, we report on the transformation of androst-4-ene-3,17-dione (**1**), pregnenolone (**2**), DHEA (**3**) and estradiol (**4**) by *Gelasinospora retispora*.

2. Experimental

2.1. Materials and methods

NMR spectra were recorded on a JEOL α-500 FT NMR spectrometer (^{1}H; 500 MHz, ^{13}C; 125 MHz) with TMS as an internal standard. The mass spectra were performed on a HITACHI M2500. Gas chromatographic analyses were performed on a Shimadzu GC-2010 using DB1 (0.25 mm × 30 m, 0.25 μm) capillary column. The IR spectra were measured on a BIO-RAD FTS3000. Melting points were measured using a Yanaco micro melting point apparatus. HPLC was performed on a JASCO 880 PU (pump) and 830 RI (refractometer) using the normal phase LiChrosorb Si-60 (7 μm) column and the reversed phase YMC Hydrosphere C-18 (5 μm) column. Androst-4-

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ene-3,17-dione, pregnenolone, DHEA and estradiol were purchased from Tokyo Chemical Industry Co., Ltd.

2.2. Microorganism and medium

Gelasinospora retispora GRK002 was provided by Professor Emeritus Naoshi Nakamura, University of the Ryukyus. Yeast and mold medium (YM medium) [0.3% yeast extract, 0.3% malt extract and 0.5% peptone in distilled water] was used for the biotransformation experiment.

2.3. Growth and biotransformation conditions

1. An Erlenmeyer flask (1000 ml) containing 300 ml of YM medium was inoculated with a suspension of *G. retispora*, and incubated at 26 °C for 4 days under shaking (90 rpm). After full growth of the microorganisms, 100 mg of substrate dissolved in 2 ml of methanol was added to the grown cultures. The incubation was then continued for 14 days at 26 °C. The culture was filtered and the broth was extracted twice with ethyl acetate (EtOAc) and concentrated in vacuo, and the crude extract was fractionated by HPLC.

2. The fungi were incubated at 26 °C in 300 ml Erlenmeyer flasks with 150 ml of YM medium. After 4 days incubation, 20 mg of DHEA (**3**) and estradiol (**4**) was added. The metabolites were extracted with EtOAc after 6 days.

Medium without *G. retispora* was also prepared to act as a control. Metabolites were not detected in the control.

2.3.1. Biotransformation of androst-4-ene-3,17-dione (*1*) at different pH values

For these experiments 200 ml Erlenmeyer flasks were used, filled with 100 ml of YM medium (pH 5–9) and inoculated with *G. retispora* at 26 °C. After 4 days incubation, substrate (**1**, 10 mg) was added to the medium and further cultivated for 6 days. After incubation, the mycelia were removed by filtration, and filtered culture was extracted with EtOAc. The extracts were concentrated in vacuo and analyzed on GC and ^{1}H NMR.

2.3.2. Transformation of androst-4-ene-3,17-dione (*1*) at the increased temperature

Each 200 ml Erlenmeyer flasks containing 100 ml of YM medium (pH 5–7) were inoculated with suspension of *G. retispora* and then incubated for 4 days at 34 °C on a rotary shaker (90 rpm). Androst-4-ene-3,17-dione (**1**, 10 mg) was added to medium. After 6 days incubation, the culture and mycelia were separated by filtration, and the filtered culture medium was extracted with EtOAc. The EtOAc extracts were analyzed on GC and ^{1}H NMR.

2.3.3. Transformation of androst-4-ene-3,17-dione (*1*) at the higher substrate loadings

To produce **5**, cultures were transferred into 1000 ml flask containing 300 ml of YM medium at 26 °C for 4 days. Androst-4-ene-3,17-dione (**1**, 500 mg or 1000 mg) was added as substrate, and the mixture was incubated for 6 days. After the incubation period, the grown culture was removed by filtration and the filtrate was extracted with EtOAc. The crude oil was analyzed by GC and ^{1}H NMR.

2.4. Biotransformation of androst-4-ene-3,17-dione (*1*)

The crude extract obtained from the biotransformation of **1** was separated by normal phase HPLC (*n*-hexane–EtOAc, 1:3) to give two fractions. The first fraction was purified by HPLC (*n*-hexane–EtOAc, 1:15) to afford **5**.

Table 1
^{13}C NMR data for transformation products determined in $CDCl_3$ or CD_3OD[a].

Carbon atom	Compounds						
	3	**5**	**6**[a]	**7**	**8**	**9**	**10**[a]
1	37.2	37.4	38.6	37.2	35.8	38.2	127.1
2	31.6	33.3	32.3	31.6	31.0	31.3	116.0
3	71.6	199.9	72.4	71.6	71.2	70.6	156.3
4	42.2	124.8	43.0	42.2	41.9	42.4	117.5
5	141.0	170.0	142.3	141.4	146.6	165.7	140.1
6	120.9	34.1	122.3	120.5	123.6	125.3	68.3
7	30.8	30.2	32.6	30.4	64.3	200.2	27.2
8	31.5	34.5	33.3	27.6	37.2	43.6	34.3
9	50.2	59.2	51.9	50.6	42.6	55.7	45.4
10	36.6	40.0	37.8	36.8	37.5	35.7	132.7
11	20.4	68.7	21.8	20.3	20.1	68.3	37.6
12	31.4	42.9	37.9	32.7	31.3	41.9	38.0
13	47.5	47.9	43.8	46.7	47.1	48.0	44.6
14	51.8	50.0	52.7	55.9	44.9	44.5	50.9
15	21.9	21.7	24.4	67.3	21.9	23.8	23.9
16	35.9	35.7	30.6	46.7	36.9	35.7	30.7
17	221.2	218.4	82.5	223.5	221.1	200.2	82.5
18	13.5	14.6	11.5	17.5	13.3	14.5	11.7
19	19.4	18.3	19.9	19.3	18.3	17.1	–

2.4.1. 11α-Hydroxyandrost-4-ene-3,17-dione (*5*) [18]

White plates; m.p. 196–197 °C; IR (KBr): ν_{max} cm^{-1} 3445, 1738, 1667; ^{1}H NMR ($CDCl_3$) δ 5.76 (1H, *s*, H-4), 4.08 (1H, *ddd*, *J* = 15.0, 10.5, 5.0 Hz, H-11), 1.35 (3H, *s*, H-19), 0.95 (3H, *s*, H-18); ^{13}C NMR ($CDCl_3$) see Table 1; MS (EI) *m*/*z* 302 [M]$^+$.

2.5. Biotransformation of pregnenolone (*2*)

The EtOAc extract was separated by normal phase HPLC (*n*-hexane–EtOAc, 3:2) to afford **3**, **6**, and **7**.

2.5.1. 3β-Hydroxyandrost-5-en-17-one (*3*) [19]

White needles; m.p. 148–149 °C; IR (KBr): ν_{max} cm^{-1} 3445, 1739, 1650; ^{1}H NMR ($CDCl_3$) δ 5.42 (1H, *d*, *J* = 5.0, H-6), 3.55 (1H, *m*, H-3), 1.05 (3H, *s*, H-19), 0.90 (3H, *s*, H-18); ^{13}C NMR ($CDCl_3$) see Table 1; MS (EI) *m*/*z* 288 [M]$^+$.

2.5.2. 3β,17β-Dihydroxyandrost-5-ene (*6*)

White plates; m.p. 174–176 °C; IR (KBr): ν_{max} cm^{-1} 3448, 1653; ^{1}H NMR (CD_3OD) δ 5.25 (1H, *d*, *J* = 5.0, H-6), 3.47 (1H, *t*, *J* = 9.0, H-17), 3.30 (1H, *m*, H-3), 0.94 (3H, *s*, H-19), 0.65 (3H, *s*, H-18); ^{13}C NMR (CD_3OD) see Table 1; MS (EI) *m*/*z* 290 [M]$^+$.

2.5.3. 3β,15β-Dihydroxyandrost-5-en-17-one (*7*)

White plates; m.p. 172–173 °C; IR (KBr): ν_{max} cm^{-1} 3446, 1736, 1653; ^{1}H NMR ($CDCl_3$) δ 5.46 (1H, *d*, *J* = 5.5, H-6), 4.62 (1H, *t*, *J* = 5.0, H-15), 3.61 (1H, *m*, H-3), 1.25 (3H, *s*, H-18), 1.14 (3H, *s*, H-19); ^{13}C NMR ($CDCl_3$) see Table 1; MS (EI) *m*/*z* 304 [M]$^+$.

2.6. Conversion of DHEA (*3*)

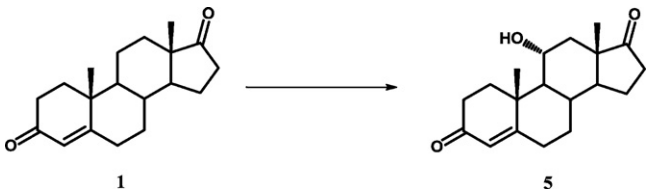
The transformation products were separated by HPLC (*n*-hexane–EtOAc, 1:5) to give **8** and **9**.

2.6.1. 3β,7α-Dihydroxyandrost-5-en-17-one (*8*) [20,21]

White plates; m.p. 180–181 °C; IR (KBr): ν_{max} cm^{-1} 3390, 1734, 1643; ^{1}H NMR ($CDCl_3$) δ 5.65 (1H, *dd*, *J* = 5.4, 1.95, H-6), 3.96 (1H, *br s*, H-7), 3.59 (1H, *m*, H-3), 1.02 (3H, *s*, H-19), 0.89 (3H, *s*, H-18); ^{13}C NMR ($CDCl_3$) see Table 1; MS (EI) *m*/*z* 304 [M]$^+$.

2.6.2. 3β,11α-Dihydroxyandrost-5-ene-7,17-dione (*9*)

White plates; m.p. 129–131 °C; IR (KBr): ν_{max} cm^{-1} 3440, 1735, 1656; ^{1}H NMR $CDCl_3$) δ 5.80 (1H, *s*, H-6), 4.18 (1H, *ddd*, *J* = 15.0,



Scheme 1. Biotransformation of **1** by *G. retispora*.

10.0, 5.0 Hz, H-11), 3.70 (1H, *m*, H-3), 1.37 (3H, *s*, H-19), 0.91 (3H, *s*, H-18); ^{13}C NMR ($CDCl_3$) see Table 1; MS (EI) *m*/*z* 318 $[M]^+$.

2.7. Transformation of estradiol (**4**)

The crude extract was separated using normal phase HPLC (*n*-hexane–EtOAc, 1:5) to give four fractions. The first fraction was purified by reversed phase HPLC (MeOH–H_2O, 20:1) to give **10**.

2.7.1. 6β-Hydroxyestradiol (**10**) [22]

White plates; m.p. 137–139 °C; IR (KBr): ν_{max} cm^{-1} 3430, 3210; ^{1}H NMR (CD_3OD) δ 7.14 (1H, *d*, *J* = 8.6, H-1), 6.78 (1H, *d*, *J* = 2.7, H-4), 6.67 (1H, *dd*, *J* = 8.6, 2.7, H-2), 4.63 (1H, *dd*, *J* = 3.9, 2.2, H-6), 3.66 (1H, *t*, *J* = 8.5, H-17), 0.80 (3H, *s*, H-18); ^{13}C NMR (CD_3OD) see Table 1; MS (EI) *m*/*z* 288 $[M]^+$.

3. Results and discussion

The biotransformation result of **1** was summarized in Scheme 1. The mixtures of compounds were obtained, but only the major products were isolated and identified. The transformation of **1** using *G. retispora* for 14 days gave 11α-hydroxyandrost-4-ene-3,17-dione (**5**) in a 81% yield. The structure of **5** was determined by its IR, EIMS, ^{1}H NMR and ^{13}C NMR spectra.

The mass of molecular ion ($[M]^+$ *m*/*z* 302) of **5** is 16 large from that of **1**, which indicates hydroxylation of the substrate. The ^{1}H NMR spectrum showed a methine proton due to the secondary alcohol at δ_H 4.08 (*ddd*, *J* = 15.0, 10.5, 5.0 Hz), which correlated with H-9 and H-12 in the COSY spectrum. The stereochemistry of the hydroxyl group was established using NOE correlations. NOE enhancement between Me-18 and H-11β indicated that the hydroxyl group is α configuration (Fig. 1). Moreover, the multiplet of the signal at δH 4.08 (*ddd*, *J* = 15.0, 10.5, 5.0 Hz, H-11) in the ^{1}H NMR spectrum confirmed equatorial configuration which is consist with a 11α-hydroxyl group. The spectral data were in agreement with literature data [18].

From these results, it seems that **1** was easily hydroxylated at C-11α position by *G. retispora*. After 1 day transformation of **1**, the yield of **5** was 3%, and after additional 5 days reached 81% (Fig. 2). It is known that some fungi converted **1** to C-14α [21,23,24], C-11α [25,26] and C-15α [27,28] hydroxyl derivatives, and most of these fungi gave some hydroxyl products in conversion of **1**. In this study,

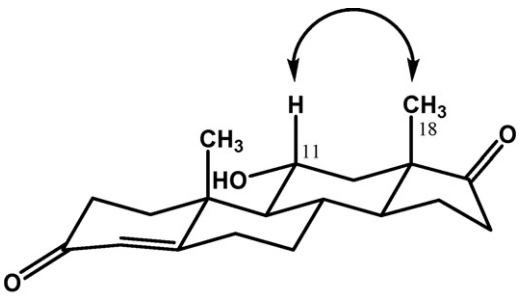


Fig. 1. NOE observed in the NOE difference experiments of **5**.

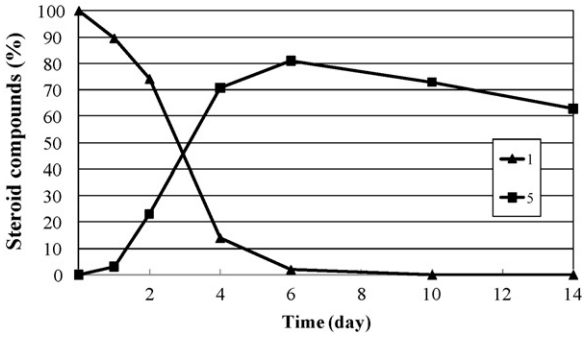


Fig. 2. Time course of biotransformation of **1** by *G. retispora*.

G. retispora afforded **5** which hydroxylated at C-11α position of **1** preferentially.

The effect of pH on the transformation of **1** to **5** was studied (Fig. 3). The results suggested that: (i) in each pH, **5** was obtained in about 80% yields; (ii) the pH with the best yield was 6, about 89%. In addition, to examine the effect of temperature, conversion of **1** was performed at pH 5–7 and 34 °C. As a result, yield of **5** was the highest at pH 5 in 98%. At pH 6–7, **5** was obtained in high yield of 90% or more. Based on these results, the best condition to produce **5** is at pH 5 and temperature of 34 °C.

G. retispora used for this experiment was isolated from the soil of sugarcane field. It is known that the optimal pH and temperature for sugarcane growing up are pH 6–7 and about 32 °C [29,30], these conditions are almost the same as the results of this experiment. From these results, it was found that the cultivation conditions of sugarcane (pH and temperature) are related to transformation by *G. retispora*.

11α-Hydroxy steroids are very useful compounds for the synthesis of pharmacologically active compounds. Therefore, to investigate whether C-11α hydroxylation by *G. retispora* can be industrially used, the experiments at the increased substrate conditions were performed. For 0.5 g (1.7 mg/ml) of **1**, the yield of **5** was 75%, on the other hand the yield was 46% at the higher substrate loading of 1.0 g (3.3 mg/ml). From these results, it was found that **5** is obtained in high yield even if amount of substrate was increased. However, 1.7 mg/ml or less of the concentration of sub-

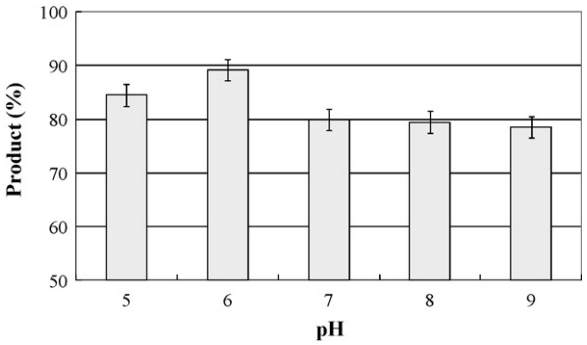
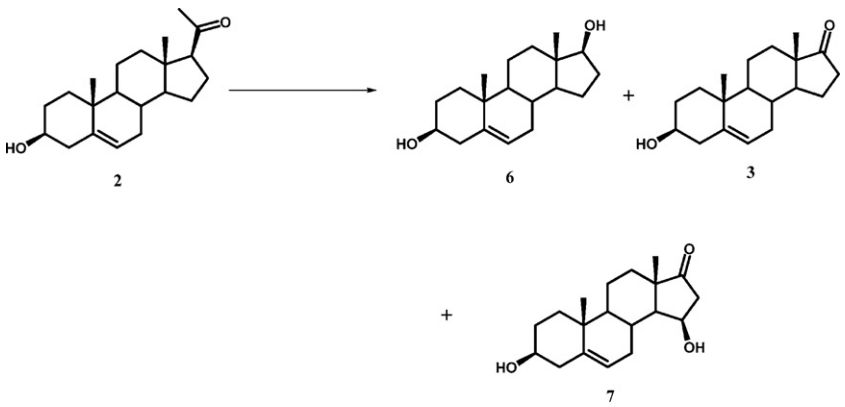


Fig. 3. Biotransformation of **1** at different pH values.



Scheme 2. Biotransformation of **2** by *G. retispora*.

strate is preferable. In this study, it seems that conversion of **1** by *G. retispora* is possible using substrate at the loading ranging from 0.33 to 3.3 mg/ml.

Transformation of **2** afforded three products: DHEA (**3**), 3β,17β-dihydroxyandrost-5-ene (**6**) and 3β,15β-dihydroxyandrost-5-en-17-one (**7**) (Scheme 2). Their structures were determined by analysis using ^{1}H NMR, ^{13}C NMR, IR and EIMS. The spectral data of **3** were in agreement with those reported in the literature [19].

The ^{1}H and ^{13}C NMR spectra of **6** were similar to that of **3**. In the ^{1}H NMR an additional methine proton due to the secondary alcohol at δ_H 3.47 (*t*, *J* = 9.0 Hz) was observed, which correlated with H-16 in the COSY spectrum. ^{13}C NMR spectrum lacked carbonyl carbon, which was replaced by a methine (δ_C 82.5), suggesting a simple reduction of carbonyl group as structure **6**. A triplet at δ_H 3.47 showed in the spectrum, which is typical for 17β-hydroxy steroids. A NOE correlation from H-16α to H-17α confirmed the β configuration of the 17-OH.

The mass spectrum ([M]$^{+}$ *m*/*z* 304) of **7** indicates hydroxylated product of **3**. The ^{1}H NMR spectrum showed a secondary alcohol methine proton at δ_H 4.62 (*t*, *J* = 5.0 Hz), which correlated with H-14 and H-16 in COSY spectrum. NOE enhancement between H-14 and H-15 indicated that the new hydroxyl group is β.

The time course of **2** to the metabolites **3**, **6** and **7** was summarized in Fig. 4. The results showed that: (i) after 4 days incubation, amount of the substrate decreased slowly; (ii) metabolite **6** was obtained as the major product, about 18%, after which it decreased; (iii) the yield of **3**, reached the maximal amount, about 23%, after 14 days incubation; (iv) the minor metabolite **7** was not obtained after 6 days, and then **7** increased and the final yield was about 11%.

In the reaction pathway of **2**, it is considered that side-chain was cleaved by Baeyer–Villiger oxidation. The Baeyer–Villiger reaction by many fungi (e.g. of genera *Fusarium*, *Aspergillus*, *Penicillium*) has been studied in detail with steroids, and side-chain cleavage of pregnenolone by Baeyer–Villiger oxidation has been reported [31]. It seems that oxidation of **2** progresses by following reactions (Scheme 3): oxidation of 17β-acetyl chain to 17β-acetoxy-3β-hydroxyandrost-5-ene; ester bond hydrolyzes leading to 17β-hydroxyl compound **6** and then hydroxylation at C-15β position to **7** and oxidation to 17-oxo product **3**.

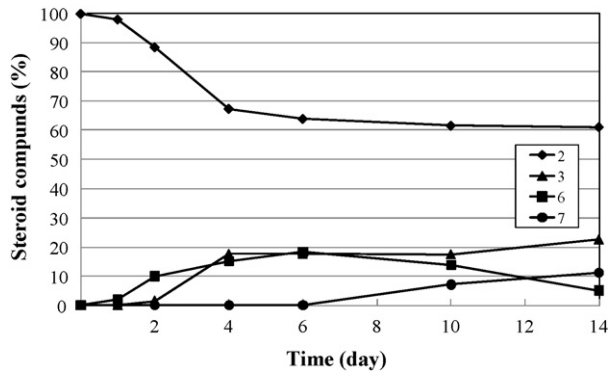


Fig. 4. Time course of biotransformation of **2** by *G. retispora*.

There are some reports on side-chain cleavage [32] or hydroxylation using microorganisms. However, most of microorganisms perform only one of reactions. In this study, *G. retispora* was carried out both side-chain cleavage and hydroxylation.

Conversion of **3** gave two hydroxyl compounds; 3β,7α-dihydroxyandrost-5-en-17-one (**8**, 35%) and 3β,11α-dihydroxyandrost-5-ene-7,17-dione (**9**, 61%) (Scheme 4).

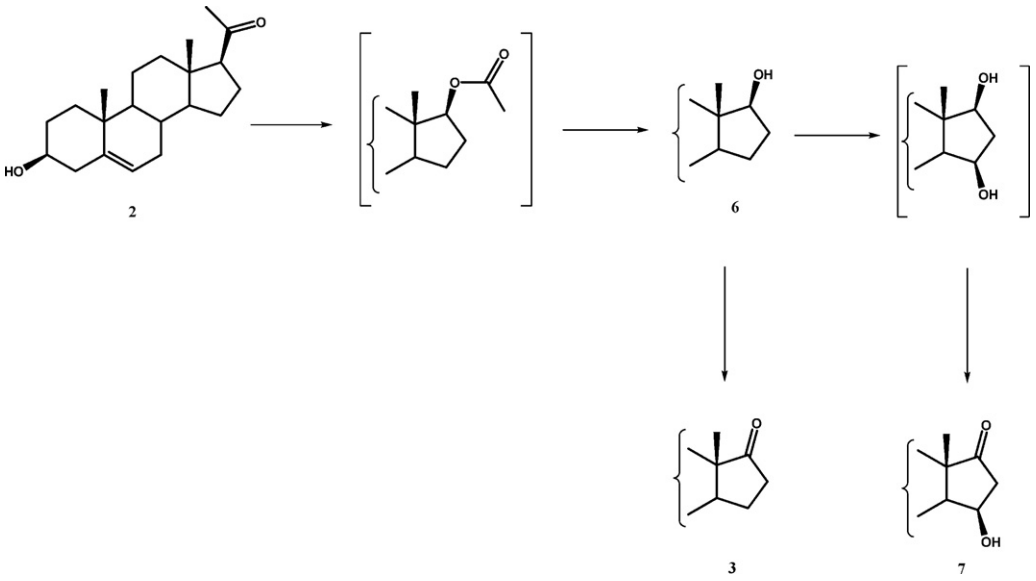
The ^{1}H and ^{13}C NMR spectra of **8** were similar to that of **7**. The signal at δ_H 3.96 (*br s*) correlated with the signal of a vinyl proton at C-6 in COSY spectrum. NOE correlation from H-7 to H-8 confirmed the α configuration of 7-OH. The spectral data for 3β,7α-dihydroxyandrost-5-en-17-one (**8**) are in agreement with the literature [18,19].

The ^{13}C NMR spectrum of **9** showed new carbonyl carbon at δ_C 200.2. The singlet of a vinyl proton at C-6 (δ_H 5.80) indicates the presence of a neighboring quarternary carbon. The HMBC correlations of H-8 and H-9 to the carbonyl carbon confirmed the position of the carbonyl group at C-7. In the ^{1}H NMR spectrum an additional secondary alcohol signal at δ_H 4.11 was observed, which correlated with H-9 and H-12 in COSY spectrum. These spectral data showed that the hydroxyl group attached at C-11. The OH stereochemistry was determined as α configuration by the NOE's experiment; irradiation of the signal at δ_H 0.91 (Me-18) enhanced the signal at δ_H 4.11 (H-11).

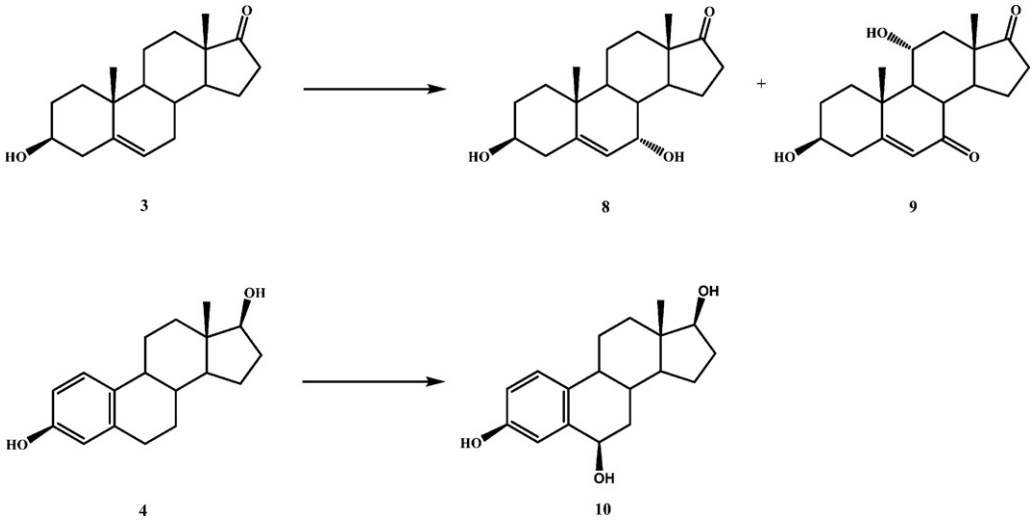
The oxidation of DHEA using fungi (e. g. *Mucor pififormis*, *Fusarium moniliforme*, *Rhizopus* sp.) has been studied about C-7 position mainly. In this study, it was found that DHEA (**3**) was hydroxylated by *G. retispora* at C-7α and C-11α position (Scheme 4). After 2 days of DHEA transformation, the 7α-hydroxy derivative (**8**) was obtained, and then 11α-hydroxy derivative (**9**) was obtained. 7-Oxygenated derivatives of DHEA can have effects in the immune system [33] and 11α-hydroxy product is used as an intermediate in the synthesis of a contraceptive drug [34]. The hydroxylation of DHEA (**3**) is important to the synthesis of various therapeutic drugs, and it seems that this study develops more by using *G. retispora* which was not used as biocatalyst until now.

Transformation of estradiol (**4**) afforded 6β-hydroxy product **10** in about 77% yield (Scheme 4).

The ^{1}H and ^{13}C NMR spectra data of **10** were similar to those of **4**. In the ^{1}H NMR spectrum, an additional signal of a methine proton due to the new secondary alcohol at δ_H 4.63 appeared, which was



Scheme 3. Reaction pathway of **2**.



Scheme 4. Transformation of **3** and **4** by *G. retispora*.

correlated with C-5 and C-8 position in HMBC spectrum. Additionally, the signal of vinyl proton at δ_H 6.78 (H-4) was correlated with C-6 position in HMBC spectrum. These spectral data suggested the existence of a hydroxyl group at C-6. The multiplet of the signal at δ_H 4.63 (*dd*, *J* = 3.9, 2.2 Hz, H-6) in the ^{1}H NMR spectrum was confirmed as axial configuration which is consist with a 6β-hydroxyl group.

The hydroxylation of **4** has been studied for 2-hydroxyestradiol and 4-hydroxyestradiol possessing putative carcinogenic metabolites [35]. It is known that these reactions are catalyzed by cytochrome P450, and it is considered that cytochrome P450 is related about the hydroxylation of estradiol (**4**) at C-6 position by *G. retispora*.

4. Conclusion

The biotransformation of androst-4-ene-3,17-dione (**1**) using *Gelasinospora retispora* gave compound **5** that was hydroxylated at C-11α position with good yield. In the case of pregnenolone (**2**), the products with cleaved the side-chain, namely, DHEA (**3**), 3β17β-dihydroxyandrost-5-ene (**6**) and 3β,15β-dihydroxyandrost-5-en-17-one (**7**) were obtained. Conversion of DHEA (**3**) and estradiol (**4**) resulted in the formation of products hydroxylated at positions C-7α, C-11α and C-6β position, respectively. It was found that **1** was hydroxylated at C-11α position preferentially at pH 5 and 34 °C, and in the case of **2**, the side-chain was cleaved followed by hydroxylation at C-15β using *G. retispora*.

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References

[1] P. Fernandes, A. Cruz, B. Angelova, H.M. Pinheiro, J.M.S. Cabral, Enzyme Microb. Technol. 32 (2003) 688–705.
[2] D.H. Peterson, H.C. Murray, J. Am. Chem. Soc. 74 (1952) 1871–1872.
[3] J. Fried, R.W. Thoma, J.R. Gerke, J.E. Herz, M.N. Donim, D. Perlman, J. Am. Chem. Soc. 74 (1952) 3962–3963.
[4] P.S. Hench, E.C. Kendall, C.H. Slocumb, H.F. Polley, Proc. Staff Meetings Mayo Clin. 24 (1949) 181.
[5] S.B. Mahato, S. Garai, Steroids 62 (1997) 332–345.

[6] D. Perlman, E. Titus, J. Fried, J. Am. Chem. Soc. 74 (1952) 2126.
[7] R.M. Dodson, A.H. Goldkamp, R.D. Muir, J. Am. Chem. Soc. 79 (1957) 3921.
[8] H.L. Herzog, M.J. Gentles, E.B. Hershberg, F. Carvajal, D. Sutter, W. Charney, C.P. Schaffner, J. Am. Chem. Soc. 79 (1957) 3921–3922.
[9] G. Greenspan, C.P. Schaffner, W. Charney, H.L. Herzog, E.B. Hershberg, J. Am. Chem. Soc. 79 (1957) 3822–3923.
[10] W. McAleer, M. Kozlowski, T. Stoudt, J. Chemerda, J. Org. Chem. 23 (1958) 508.
[11] G. Defaye, M.J. Luche, E.M. Chambaz, J. Steroid Biochem. 9 (1978) 331–336.
[12] M.V. Donova, O.V. Egorova, V.M. Nikolayeva, Process Biochem. 40 (2005) 2253–2256.
[13] A. Gubler, Ch. Tamm, Helv. Chim. Acta 41 (1958) 297–301.
[14] Ch. Tamm, A. Gubler, G. Juhasz, E. Weiss-Berg, W. Züercher, Helv. Chim. Acta 46 (1963) 889–906.
[15] A. Al-Aboudi, M.Y. Mohammad, S. Haddad, R. Al-Far, M.I. Choudhary, Atta-ur-Rahman, Steroids 74 (2009) 483–486.
[16] D. Kelly, S. Kelly, Nat. Biotechnol. 21 (2003) 133–134.
[17] R.C. Tweit, E.A. Brown, S. Kraychy, S. Mizuba, R.D. Muir, R.T. Nicholson, Chem. Pharm. Bull. 12 (1964) 859–865.
[18] M.I. Choudhary, S. Sultan, M.T.H. Khan, A. Yasin, F. Shaheen, Atta-ur-Rahman, Nat. Prod. Res. 18 (6) (2004) 529–535.
[19] H. Li, H.M. Liu, W. Ge, L. Huang, L. Shan, Steroids 70 (2005) 970–973.
[20] T. Kołek, J. Steriod Biochem. Mol. Biol. 71 (1999) 83–90.
[21] T. Janeczko, J. Dmochowska-Gładysz, E. Kostrzewa-Susłow, A. Białońska, Z. Ciunik, Steroids 74 (2009) 657–661.
[22] E.P. Burrows, D.L. Di Pietro, H.E. Smith, J. Org. Chem. 37 (25) (1972) 4000–4002.
[23] V.V. Kollerov, A.A. Shutov, V.V. Fokina, G.V. Sukhodol'skaya, M.V. Donova, J. Mol. Catal. B: Enzym. 55 (2008) 61–68.
[24] M.A. Faramarzi, M. Aghelnejad, M.T. Yazdi, M. Amini, N. Hajarolasvadi, Steroids 73 (2008) 13–18.
[25] Z. Xiong, Q. Wei, H. Chen, S. Chen, W. Xu, G. Qiu, S. Liang, X. Hu, Steroids 71 (2006) 979–983.
[26] A.C. Hunter, P.W. Mills, C. Dedi, H.T. Dodd, J. Steroid Biochem. Mol. Biol. 108 (2008) 155–163.
[27] T. Kołek, A. Świzdor, J. Steroid Biochem. Mol. Biol. 67 (1998) 63–69.
[28] M.I. Choudhary, S. Sultan, M.T.H. Khan, A. Yasin, F. Shaheen, Atta-ur-Rahman, Nat. Prod. Res. 18 (2004) 529–535.
[29] K. Oya, K. Kina, Sci. Bull. Fac. Agric. Univ. Ryukyus 36 (1989) 25–31.
[30] T. Terauchi, M. Matsuoka, M. Kobayashi, H. Nakano, Jpn. J. Trop. Agric. 43 (1999) 18–25.
[31] T. Kołek, A. Szpineter, A. Świzdor, Steroids 73 (2008) 1441–1445.
[32] M. Vitas, D. Rozman, R. Komel, S.L. Kelly, Phytochemistry 40 (1995) 73–76.
[33] S. Chalbot, C. Trap, J.P. Monin, R. Morfin, Steroids 67 (2002) 1121–1127.
[34] A. Jellel, E. Ilkőy, G. Horváth, I. Pallagi, J. Sütő, G. Ambrus, J. Mol. Catal. B: Enzym. 5 (1998) 385–387.
[35] J. Williamson, D. Van Orden, J.P. Rosazza, Appl. Environ. Microbiol. 49 (1985) 563–567.