# Biotransformation XLVII: transformations of 5-ene steroids in *Fusarium culmorum* culture

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## Abstract

The course of the transformation of six 5-ene steroids with varying substituents at C-17 or/and C-3: dehydroepiandrosterone (DHEA), 5-androsten-3β,17β-diol, 17α-methyl-5-androsten-3β,17β-diol, 5-androsten-17-one, 5-androsten-3β-ol and pregnenolone by *Fusarium culmorum* was investigated. Three substrates with oxygen functions at C-3 and C-17 i.e. DHEA, 5-androsten-3β,17β-diol and 17α-methyl-5-androsten-3β,17β-diol were hydroxylated entirely at 7α-axial, allylic position. The mixture of 7α-hydroxy- and 7α,15α-dihydroxyderivatives was formed during the transformation of pregnenolone and 5-androsten-17-one, from the latter 2α,7α-dihydroxyderivative was also obtained. 7α,15α- Dihydroxyderivative was the only product isolated from the 5-androsten-3β-ol post-transformation mixture. The time-course of the DHEA transformation by *F. culmorum* shows that the substrate induces 7α-hydroxylase activity. DHEA was transformed by androstenedione induced *F. culmorum* cultures to a larger extent than by a noninduced microorganism; the selectivity of the transformation remained unchanged. © 1999 Elsevier Science Ltd. All rights reserved.

*Keywords:* Microbial hydroxylation; *Fusarium culmorum*; 5-ene steroids

## 1. Introduction

The microbial hydroxylation of steroids has been studied extensively, mainly for pharmacological reasons [1–3]. Most of the studies were carried out on hydroxylation of 4-ene-3-oxo and 5α saturated steroid substrates with one or two oxygen functions [4–6], much less attention has been directed to microbiological hydroxylation of 5-ene steroids [4–6].

The present study has been focused on the course of transformation of 5-ene steroids with different substituents at C-17 or C-3 by *F. culmorum*.

The most recent reports on biological activity of 5-ene (C-19 and C-21) steroids [7–9] and their hydroxyderivatives [9,10] encouraged us to investigate their microbiological transformations. Some results indicate that DHEA 7-hydroxyderivatives are able to increase the immune response in mice to a larger extent than

DHEA itself [9]; biological activity of 7-hydroxylated metabolites of pregnenolone was also observed [9,10].

The introduction of oxygen function at C-7 was the most common reaction in the examples of 5-ene-steroid microbial hydroxylation described in literature [11–16]. 7α-Hydroxyderivative was the main product of DHEA transformation by *Fusarium* sp., and the mixture of 7α-hydroxy-DHEA, 7β-hydroxy-DHEA and 3β-hydroxy-5-androsten-7,17-dione was formed during the transformation of the same substrate by *Rhizopus* sp.[11]. Products of C-7 oxidation (7α-,7β-hydroxy and 7-oxoderivatives) were obtained from DHEA transformation by *Mucor piriformis*, the same strain converted pregnenolone to the mixture of 7α-hydroxy and 7α,11α-dihydroxyderivatives [12]. Oxidation at the allylic position and epoxidation of the double bond were observed during conversion of DHEA, 5-androsten-3β-ol and 5-androsten-17-one by *Cunninghamella elegans* [13]. DHEA transformation by *Fusarium moniliforme* has been studied extensively [16]. The investigated strain hydroxylated DHEA at 7α-pos-

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ition with high yield (86%). It has been found that the enzyme complex capable of hydroxylating the substrate is present in microsome fraction and contains cytochrome P-450. Enzymatic activity of 7α-hydroxylase was evidently substrate induced.

The aim of the present study was to investigate the ability of *F. culmorum* to hydroxylate 5-ene steroids and to determine the influence of the substrate structure on the reaction route. The previous study [17] showed the ability of *F. culmorum* to carry out regio- and stereospecific hydroxylation of steroid hormones and their derivatives.

## 2. Materials and methods

### 2.1. Microorganism

*F. culmorum* was obtained from the collection of the Institute of Biology and Botany, Medical University of Wrocław.

### 2.2. Conditions of cultivation and transformation

1. The fungi were incubated in 3% glucose and 1% peptone, and shaken at 20°C in 300 ml Erlenmeyer flasks with 80 ml of medium. After three days of growth, the substrate (20 mg) dissolved in 1 ml of acetone was added. The products were extracted with chloroform after 4, 8, 12, 24 or 72 h.
2. A two-day-old culture (prepared as described in 1) was supplemented with 2 mg of androstenedione and 20 mg of DHEA was added after 24 h. The products were extracted with chloroform 4 h later.

### 2.3. Preparation of acetates

1. 40 mg of substrate was dissolved in 1 ml of dry pyridine and 0.2 ml of acetic anhydride was added. The mixture was left overnight at room temperature. Next, the reaction mixture was diluted with water and the products were extracted with chloroform. The extract was washed with water, sodium carbonate solution, and then with water and dried with $MgSO_4$.
2. 25 mg of 3β,7α,15α-trihydroxy-5-pregnen-20-one 3,15-diacetate (**12a**) was dissolved in 1 ml of dry pyridine and 1 drop of acetyl chloride dissolved in 0.5 ml of pyridine was added. After 1h the reaction mixture was treated as described above.

### 2.4. Product analysis

The composition of crude mixtures was analysed by

Table 1
Composition of crude mixture obtained in DHEA transformation

| Compounds present in mixture (%) | Time of substrate incubation | | | | |
| --- | --- | --- | --- | --- | --- |
| | 4 h[a] | 4 h | 8 h | 12 h | 24 h |
| DHEA (**1**) | 77 | 96 | 73 | 24 | – |
| 7α-hydroxy-DHEA (**7**) | 20 | 4 | 23 | 71 | 96 |

[a] Transformation by androstenedione-induced culture.

TLC and GLC. TLC was carried out using silica-gel 60 plates with hexane–acetone (2:1, or 1:1 v/v) as eluent. Steroids were detected on plates with $H_2SO_4$-EtOH (1:1 v/v). The products were separated chromatographically on silica gel 0.05–0.2 (Merck) with hexane–acetone mixture (2:1v/v) as eluent.

GLC analysis was performed using a Hewlett Packard 5890A Series II gas chromatograph; HP-5 (cross-linked 5% Ph-Me-Silicone) 30 m/0.53 mm/0.88 μm film thickness (column 240° $min^{-1}$, 5–300°C $min^{-1}$).

$^1$H-NMR spectra were obtained in $CDCl_3$ using a Bruker Avance DRX 300, 300 MHz spectrometer.

## 3. Results

The yield of products was determined using GLC analysis of the crude mixture. The structure of the products after separation on column chromatography was assigned based on $^1$H-NMR spectra.

The transformations were carried out for three days with the exception of DHEA, the transformation of which was complete after 24 h; the extent of the reaction after 24 h of incubation for the remaining substrates was too small to obtain metabolites in the amount sufficient for structure elucidation. The composition of the mixtures formed after 4, 8, 12 and 24 h was investigated in order to estimate the time-course of the DHEA reaction. The transformations of DHEA by androstenedione induced and noninduced *F. culmorum* cultures were also carried out to find if androstenedione used as an inductor is able to influence the specificity of DHEA hydroxylation. Previous results [17] showed that androstenedione induced 6β- and 15α-hydroxylase(s) activities in *F. culmorum*.

### 3.1. Compounds

#### 3.1.1. Transformation of DHEA (**1**)

7α-Hydroxy-DHEA (**7**) was the only product formed during 4 h transformation of DHEA by androstenedione induced *F. culmorum* and during 4, 8 or 12 h transformation of this substrate by noninduced microorganism. Table 1 shows the percentage of **7** in the

Table 2
¹H-NMR data *Fusarium culmorum* transformation products and their acetates[a]

| Compound | 6-H | 3α-H | 7β-H | C*H*OR | 18-H₃ | 19-H₃ | Other significant signals |
|---|---|---|---|---|---|---|---|
| 3β,7α-dihydroxy-5-androsten-17-one (7) | 5.62 d, $J = 5,3$; 5.64^b | 3.56 m, $W_h = 22$ Hz; 3.56^b | 3.95 m, $W_h = 11$ Hz; 3.96^b | — | 0.87 s [0.89] 0.88^b | 1.00 s [1.03] 1.04^b | — |
| 3β,7α-diacetoxy-5-androsten-17-one (7a) | 5.62 d, $J = 5$ | 4.68 m, $W_h = 25$ Hz | 5.12 t, $J = 4.2$ | — | 0.87 s [0.92] | 1.05 s [1.08] | 2.03 s (6H-acetate groups) |
| 5-androsten-3β,7α,17β-triol (8) | 5.61 d, $J = 5$; 5.65^b | 3.57 m, $W_h = 23$ Hz; 3.62^b | 3.85 m, $W_h = 11$ Hz; 3.88^b | 3.71 t $J = 9$ (17α-H); 3.75^b | 0.77 s [0.80] 0.80^b | 1.01 s [1.04] 1.04^b | — |
| 17α-methyl-5-androsten-3β,7α,17β-triol (9) | 5.61 d, $J = 5.3$ | 3.58 m, $W_h = 22$ Hz | 3.85 m, $W_h = 12$ Hz | — | 0.88 s [0.88] | 1.02 s [1.03] | 1.27 s (17α-CH₃) |
| 3β,7α-dihydroxy-5-pregnen-20-one (10) | 5.61 d, $J = 5.1$; 5.61^b | 3.58 m, $W_h = 24$ Hz; 3.58^b | 3.87 m, $W_h = 11$ Hz; 3.86^b | — | 0.64 s [0.64] 0.64^b | 1.00 s [1.00] 1.00^b | 2.14 s (21-H₃), 2.56 t $J = 9$ (17α-H) |
| 3β,15α-dihydroxy-5-pregnen-20-one (11) | 5.36 m, $W_h = 9$ Hz; 5.4^c | 3.56 m, $W_h = 24$ Hz; 3.5^c | — | 4.10 dt $J = 8$; 3.5 (15β-H); 4.13^c | 0.66 s [0.66] 0.68^c | 1.03 s [1.02] 1.03^c | 2.17 s (21-H₃), 2.72 t $J = 9$ (17α-H) |
| 3β,7α,15α-trihydroxy-5-pregnen-20-one 3,15-diacetate (12a) | 5.58 d | 4.70 m | 3.89 m | 4.93 dt $J = 13$; 5 | 0.71 s | 1.01 s | 2.16^c; 2.8 t $J = 9.3$ (17α-H), 2.95 dt; $J = 15$; 7.5 (16β-H), 2.14 s (21-H₃), 2.03 s, 2.06 s (6H-acetate groups) |
| 3β,7α,15α-triacetoxy-5-pregnen-20-one (12b) | 5.66 d, $J = 5.4$ | 4.68 m, $W_h = 24$ Hz | Appears with 15-H, $W_h = 12$ Hz | 4.82 m (15β-H) | 0.71 s [0.72] | 1.03 s [1.03] | 2.76 t $J = 9$ (17α-H), 2.96 dt; $J = 15$; 9 (16β-H), 2.13 s (21-H₃), 1.96 s, 2.03 s, 2.05 s (9H-acetate groups) |
| 5-androsten 3β,7α,15α-triol 3,15-diacetate (13a) | 5.58 d, $J = 5.4$ | 4.57 m, $W_h = 21$ Hz | 3.91 m | 4.95 dt $J = 10$; 3.8 (15β-H) | 0.81 s [0.73] | 1.01 s [1.05] | 2.03 s, 2.04 s (6H-acetate groups) |
| 7α-hydroxy-5-androsten-17-one (14) | 5.56 d, $J = 5.2$ | — | 3.95 m, $W_h = 11$ Hz | — | 0.88 s [0.80] | 1.01 s [1.04] | |
| 2α,7α-dihydroxy-5-androsten-17-one (15) | 5.63 m, $W_h = 4.3$ Hz | — | 3.93 m | 3.87 m (2β-H) | 0.89 s [0.90] | 1.02 s [1.04] | |
| 2α,7α-diacetoxy-5-androsten-17-one (15a) | 5.61 d, $J = 7$ | — | 5.12 m, $W_h = 11$ Hz | 4.98 m $W_h = 24$ Hz (2β-H) | 0.88 s [0.93] | 1.08 s [1.13] | 2.02 s, 2.04 s (6H-acetate groups) |
| 7α,15α-dihydroxy-5-androsten-17-one 15-acetate (16a) | 5.58 d, $J = 5.2$ | — | 3.99 m | 5.02 dt $J = 10$; 3 (15β-H) | 0.96 s [0.96] | 1.03 s [1.02] | 3.2 dd $J = 20$; 8 (16β-H), 2.02 s (3H-acetate group) |

[a] Chemical shift calculated from data for substrates and individual group increments [18,21] in parentheses.
[b] Value of chemical shift according to [12].
[c] Value of chemical shift according to [20].

mixtures isolated in the experiments mentioned above. 7α-Hydroxy-DHEA (**7**) was a major product isolated (96 %), after 24 h of DHEA incubation, however, traces (< 2%) of 5-androsten-3β,7α,17β-triol (**8**) were also detected.

### 3.1.2. Transformation of 5-androsten-3β,17β-diol (2)

The transformation of 5-androsten-3β,17β-diol (**2**) gave the mixture of 50% of 7α-hydroxy-DHEA (**7**), 22% of 5-androsten-3β,7α,17β-triol (**8**) and 24% of substrate **2**.

### 3.1.3. Transformation of 17α-methyl-5-androsten-3β,17β-diol (3)

The transformation resulted in the mixture of 70% of 17α-methyl-5-androsten-3β,7α,17β-triol (**9**) and 26% of substrate **3**.

### 3.1.4. Transformation of pregnenolone (4)

Pregnenolone (**4**) transformation yielded a mixture of 15% of 3β,7α-dihydroxy-5-pregnen-20-one (**10**), 4% of 3β,15α-dihydroxy-5-pregnen-20-one (**11**), 28% of 3β,7α,15α-trihydroxy-5-pregnen-20-one (**12**), two metabolites (8% in total) of unknown structure and 40% of substrate **4**.

### 3.1.5. Transformation of 5-androsten-3β-ol (5)

The transformation gave a mixture of products, but 70% of the substrate remained unchanged. One product: 5-androsten-3β,7α,15α-triol (**13**) (10%) was isolated after the transformation of **5**.

### 3.1.6. Transformation of 5-androsten-17-one (6)

Led to a mixture of 7α-hydroxy-5-androsten-17-one (**14**) (10%), 2α,7α-dihydroxy-5-androsten-17-one (**15**) (14%), 7α,15α-dihydroxy-5-androsten-17-one (**16**) (8%) and unreacted substrate **6** (60%).

### 3.1.7. Preparation of 3β,7α,15α-trihydroxy-5-pregnen-20-one 3,15-diacetate (12a)

40 mg 3β,7α,15α-Trihydroxy-5-pregnen-20-one (**12**) was esterified and purified using column chromatography. 32 mg of 3β,7α,15α-trihydroxy-5-pregnen-20-one 3,15-diacetate (**12a**) was obtained.

### 3.1.8. Preparation of 5-androsten-3β,7α,15α-triol 3,15-diacetate (13a)

20 mg 5-androsten-3β,7α,15α-triol (**13**) was esterified and purified using column chromatography. 14 mg of 5-androsten-3β,7α,15α-triol 3,15-diacetate (**13a**) was obtained.

### 3.1.9. Preparation of 2α,7α-diacetoxy-5-androsten-17-one (15a)

Acetylation of 25 mg 2α,7α-dihydroxy-5-androsten-17-one (**15**) yielded 20 mg of 2α,7α-diacetoxy-5-androsten-17-one (**15a**).

### 3.1.10. Preparation of 7α,15α-dihydroxy-5-androsten-17-one 15-acetate (16a)

Acetylation of 22 mg 7α,15α-dihydroxy-5-androsten-17-one (**16**) gave 13 mg of 7α,15α-dihydroxy-5-androsten-17-one 15-acetate (**16a**).

### 3.1.11. Preparation of 3β,7α,15α-triacetoxy-5-pregnen-20-one (12b)

18 mg of 3β,7α,15α-triacetoxy-5-pregnen-20-one (**12b**) was obtained from 25 mg of 3β,7α,15α-trihydroxy-5-pregnen-20-one 3,15-diacetate (**12a**) added acetyl chloride.

### 3.2. Identification of metabolites

The position and configuration of the introduced hydroxy group were recognised mainly from chemical shift (Table 2) and the shape [18] of proton signals of C*H*OH (or C*H*OAc for acetates). ^{1}H-NMR spectra of triols **12**, **13** and diols **15**, **16** did not allow us to characterise precisely the C*H*OH signal shape and location due to overlapping of C*H*OH signals. Those alcohols were esterified with acetic anhydride in pyridine and the position of the introduced hydroxy group was established by spectra of acetates. The acetylation of **12**, **13** and **16** containing 7α- and 15α-hydroxy groups led to acetates **12a**, **13a** and **16a** with remaining unesterified 7α-hydroxy group (absorption band at 3555 cm$^{-1}$ in IR spectra of **12a**, **13a** and **16a** compounds exhibited the presence of hydroxy group). Similarly, 3,15-diacetate was formed during acetylation of 3β,7α,15α-trihydroxy-5-androsten-17-one [19]. Additionally, 3β,7α,15α-trihydroxy-5-pregnen-20-one 3,15-diacetate (**12a**) was treated with acetyl chloride in pyridine and 3β,7α,15α-triacetoxy-5-pregnen-20-one (**12b**) was obtained. ^{1}H-NMR spectra of the acetates obtained allowed us to determine the positions of hydroxy and acetate groups.

The presence of 6-H signal at δ: 5.36–5.66 ppm in the ^{1}H-NMR spectra of all the products shows that the double bond $C_5$–$C_6$ has been retained. Nearly all products (except **11**) contained 7α-hydroxy group — the presence of which was confirmed by a down-field shift of 6-H signal (0.22–0.25 ppm as compared to substrate) and the existence of a narrow peak of 7β-H ($W_h \approx 11$ Hz) at δ: 3.85–3.99 ppm. The peaks of 7β-H at δ: 5.12 ppm ($W_h \approx 11$ Hz) were observed in ^{1}H-NMR spectra of 7α-acetates **7a** and **15a**. A characteristic peak (in the shape of a triplet split into doublet [18]) of 15β-H at 4.1 and 0.2 ppm down-field shift (as compared to substrate) of 17α-H showed the presence of 15α-hydroxy group in **11**. The shape of 15β-H peak at δ 4.93–5.02 ppm similar to that in **11**, was observed in the spectra of **12a**, **13a** and **16a** acetates. Significant
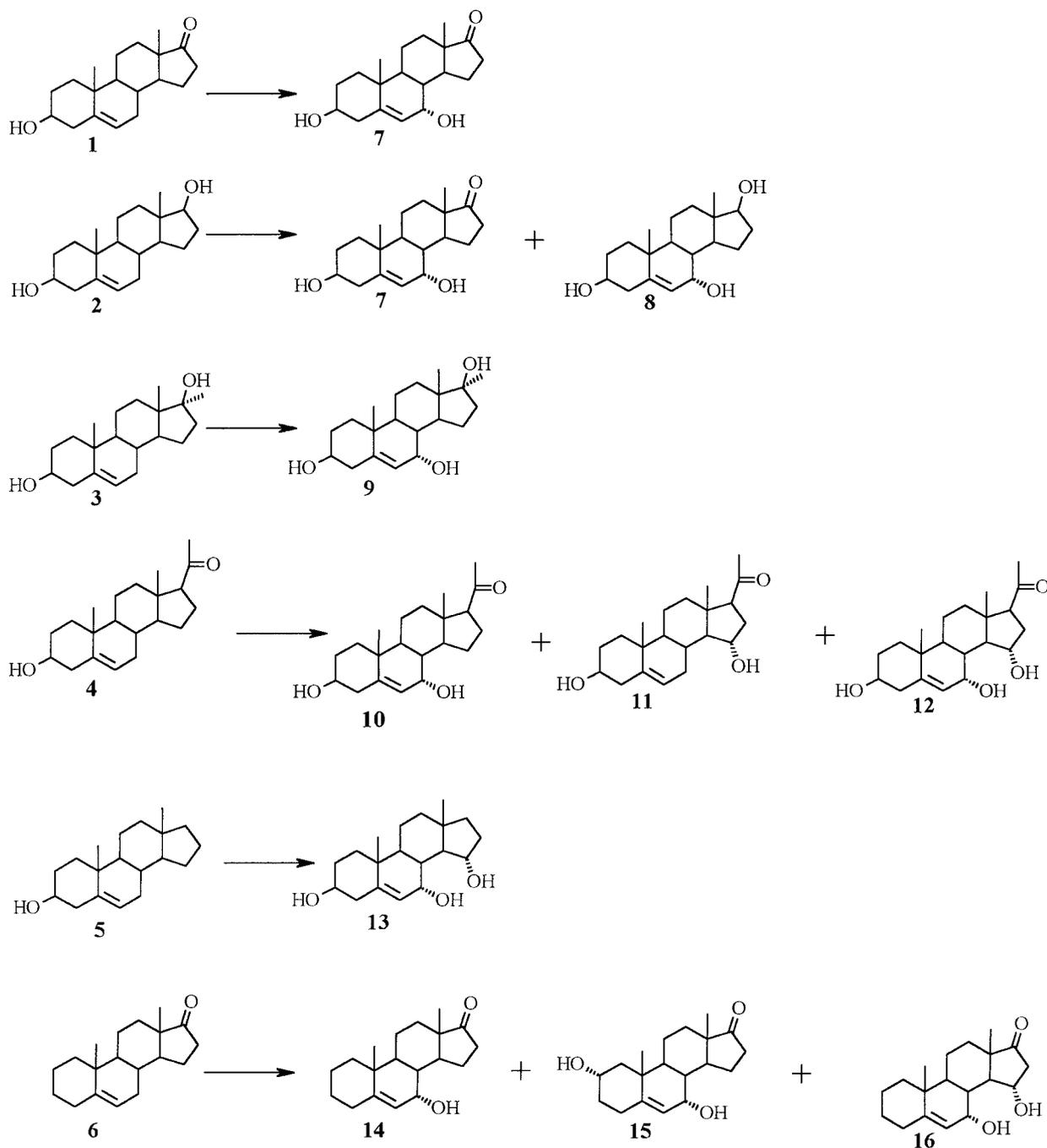
Fig. 1. The course of transformations of 5-ene steroids.

signals of 16β-proton in the spectra of **12a**, **12b** (two triplets) and **16a** (dd) proved the presence of 15α-acetoxy group. The down-field shift of 17α-H peak in the presence of 7α- or/and 15α-substituents (-OH or -OAc) was observed in the spectra of **8**, **10**, **12a** and **12b**. The values of chemical shift of significant signals of **7**, **8**, **10** [12] and **11** [20] are in agreement with those reported in literature (Table 2).

### 3.2.1. 2α,7α-dihydroxy-5-androsten-17-one (15)

The peaks at $\delta \approx 3.93$ ppm and $\delta \approx 3.87$ ppm indicated the presence of two secondary hydroxy groups, those peaks were partially overlapping, so that it was difficult to determine the position of the hydroxy groups. Chemical shift of 6-H ($\delta = 5.63$ ppm) showed the presence of allylic hydroxy group. Chemical shift $\delta = 3.87$ ppm and a broad peak profile suggested the

presence of 2α-hydroxy group [6,18]. The positions of signals of 19-H$_3$ and 18-H$_3$ in the spectrum of **15** were in accordance with those calculated [18,21] for 2α,7α-dihydroxy-5-androsten-17-one (**15**); (the differences were 0.021 and 0.009 ppm, respectively).

### 3.2.2. α,7α-diacetoxy-5-androsten-17-one (15a)

The peaks of protons of C*H*OAc in the spectrum of **15a** were clearly separated. The chemical shift of 6-H ($\delta = 5.61$ ppm) and a narrow ($W_h = 11$ Hz) peak at $\delta = 5.12$ ppm showed the presence of 7α-acetoxy group. The shape of a broad peak at $\delta = 4.98$ ppm ($W_h >= 24$ Hz) was very similar to that presented in literature [18] for peaks of 2β-, or 3α-*H*OH; but it was not a peak of 3α-HOH because diol **15** was different from 7α-hydroxy-DHEA (**7**) (Table 2). The position of peak $\delta = 4.98$ ppm was similar to that expected for this of 2β-HOAc, taking into account the chemical shift of 2β-proton in the spectrum of diol **15**. Chemical shifts of angular methyl groups were (0.045 ppm for 18-H$_3$ and 0.055 ppm for 19-H$_3$) lower, than the calculated ones [18,21], but similar differences were also observed in the **7**, **7a**, **8** and **11** (Table 2).

### 3.3. Inducibility of DHEA 7α- hydroxylase

A small (4%) amount of 7α-hydroxy-DHEA (**7**) was found after the first 4 h of reaction, after the next 4 h the amount of diol **7** increased to 23%, and after another 4 h to 70%. The comparison between the compositions of the mixtures obtained from DHEA after 4, 8 and 12 h of incubation showed that the hydroxylation rate increased with the length of time (Table 1), which proved that the presence of the substrate induced the activity of 7α-hydroxylase.

The mixture obtained after 4 h of incubation, in the culture of androstenedione induced strain, contained 20% of 7α-hydroxy-DHEA (**7**), whereas only 4% of this product was formed at the same time in the noninduced culture.

## 4. Discussion

The course of transformations of six 5-ene steroids with varying substituents at C-3 or/and C-17, i.e.: DHEA, 5-androsten-3β,17β-diol, 17α-methyl-5-androsten-3β,17β-diol, pregnenolone, 5-androsten-3β-ol and 5-androsten-17-one by *F. culmorum* were investigated (Fig. 1). All the substrates were hydroxylated in *F. culmorum* culture.

Three substrates containing oxygen functions at C-3 and C-17: DHEA, 5-androsten-3β,17β-diol, 17α-methyl-5-androsten-3β,17β-diol underwent selective hydroxylation at axial, allylic position 7α. Two products, 7α-hydroxy-DHEA (**7**) and 5-androsten-3β,7α,17β-triol

(**8**) were obtained from 5-androsten-3β,17β-diol (**2**); selective 7α-hydroxylation was observed together with partial oxidation of 17β-hydroxy group.

DHEA was transformed about 4–5 times faster than diols **2** or **3**. In a one-day transformation of DHEA the whole substrate was transformed, whereas 25% of the unreacted substrate remained after three-day reaction of diols **2** or **3**.

The presence of 17α-methyl group neither influenced the position nor rate of hydroxylation.

In addition to 7α-hydroxyderivatives, the products of 7α,15α-dihydroxylation were obtained in the transformation of pregnenolone (**4**) and 5-androsten-17-one (**6**), and 7α,15α-dihydroxyderivative **13** was the major product for 5-androsten-3β-ol (**5**). The hydroxylation of 2α-equatorial position parallel to 7α occurred mainly in the transformation of the substrate with no substituent at C-3 (5-androsten-17-one). A small amount of 15α-monohydroxyderivative **11** was also obtained from pregnenolone.

Pregnenolone, and especially monooxygenated substrates **5** and **6** were transformed slower than dioxygenated **1**, **2** and **3**.

Induction of DHEA 7α-hydroxylase activity in the presence of the substrate was observed (Table 1); the presence of androstenedione also induces the DHEA 7α-hydroxylating enzyme.

All the products obtained, except 15α-hydroxypregnenolone (**11**), carried 7α-axial, allylic hydroxy group, irrespective of the substrate structure. 7α-Hydroxylation was most frequently accompanied by 15α-hydroxylation, then dihydroxyderivatives were formed.

During the transformation of 4-ene-3oxo steroid substrates with different substituents at C-17 by the same strain, the directing effect of the substituents present in the substrate was observed [17]. The hydroxylation at 12β and 15α occurred in the presence of 4-ene-3-oxo system, but only the substrates carrying the oxygen function at C-17, were hydroxylated at 6β-position. The results obtained in transformations of six 5-ene substrates by *F. culmorum* indicate that the presence of C$_5$–C$_6$ double bond had a significant impact on the positions of hydroxylation.

Comparison of androstenedione and DHEA transformation routs indicates the impact of the substrate structure on the position of the introduced hydroxy group.

Androstenedione was hydroxylated to the mixture of 15α- and 6β-hydroxyderivatives (ratio 2:1) [17], whereas DHEA underwent selective 7α-hydroxylation. DHEA induced and noninduced *F. culmorum* cultures converted androstenedione to a similar mixture of 15α- and 6β-hydroxyderivatives, but the degree of reaction was larger in the case of induced cultures. Selective 7α-hydroxylation of DHEA occurred during transform-

ations by androstenedione induced and noninduced cultures, but the transformation was faster in the induced cultures (Table 1). The results of the androstenedione transformation by DHEA induced *F. culmorum* cultures and hydroxylation of DHEA by androstenedione induced cultures suggest that androstenedione and DHEA hydroxylations are carried out by the same enzyme complex.

The analysis of Dreiding's models shows that 6β- or 12β- and 15α-hydrogen atoms in enzyme-substrate complexes, which differ by substrate arrangement [22] could take equivalent positions towards the active site of the enzyme; this confirms the assumption that the hydroxylation of 6β- or 12β- and 15α-positions in 4-ene-3-oxo steroid substrates was carried out by the same enzyme complex. The vicinity of 6β and 7α sites and their axial, allylic feature, suggest that they may have been hydroxylated by the same enzyme. The analysis of Dreiding's models shows that the arrangement in which 6β- and 7α-hydrogen atoms were in similar positions towards the enzyme active site could not be found. The hydroxylation of 6β- and 7α-positions by the same enzyme could be associated with a step-wise radical mechanism of microbial hydroxylation [23,24]. The stereoelectron effect of Π electrones, which facilitates the abstraction of allylic, axial hydrogen and stabilises the radical being formed, is likely to affect the position of the introduced hydroxy group.

Hydroxylation of 2α-, 6α- [25], 12β- or 15α-equatorial positions and allylic, axial 4β-, 6β- or 7α- (in: 2-ene-1-oxo- [25], 4-ene-3-oxo- [17] and 5-ene-substrates, respectively) positions occurred, during the transformations of different steroid substrates by *F. culmorum*.

There are other data in literature showing a similar influence of the presence of $C_5$–$C_6$ double bond in the substrate on the position of the introduced hydroxy group. DHEA underwent selective 7α-hydroxylation by *Gibberella saubineti*, whereas testosterone, progesterone and deoxycorticosterone were hydroxylated at 15α- or 6β-positions by the same strain [14]. Similar differences were observed during DHEA and its 5α-dihydroderivative transformation by *Fusarium graminearum*; DHEA was hydroxylated at 7α- while epiandrosterone at 15α-position [15]. 7α-Hydroxypregnenolone and 15α-hydroxyprogesterone were formed when pregnenolone was incubated with mycelium or spores of marine fungus *Cladosporium herbarum* [26].

The abilities of the *F. culmorum* strain to carry out the selective hydroxylation of DHEA at 7α-position with high yield (96%) can be attractive for practical use, due to the biological activity of 7α-hydroxy-DHEA. The strains described in literature were able to carry out 7α-hydroxylation of DHEA with the yield of 36% [12] and 86% [16].

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