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# Biotransformation of 11-ketoprogesterone by Filamentous Fungus, *Fusarium lini*

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**Abstract**

Microbial transformation of 11-ketoprogesterone (**1**) by the filamentous fungi *Fusarium lini* was investigated. Biotransformation of 1 with *F. lini* resulted in the production of ten oxidative metabolites (2-11). Structures of these metabolites were deduced by spectroscopic analysis and seven of them, 5-11, were found to be new metabolites.

*Keywords:* 11-Ketoprogesterone; Steroid; Microbial transformation; Hydroxylation; Filamentous fungi; *Fusarium lini*.


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## 1. Introduction

Microorganisms are well known for their ability to catalyze practically every type of reactions in nearly all classes of natural or xenobiotic organic compounds. As a result, microorganisms and their enzymes are widely employed in the synthesis of organic compounds and modification of the structures of abundantly available prototype compounds [1]. Microbial transformations are used as a general means to synthesize steroidal drugs that are difficult to be synthesized by conventional chemical procedures.

11-Ketoprogesterone (pregn-4-ene-3,11,20-trione; $C_{21}H_{28}O_3$) (**1**) is an important regulatory hormone, which was initially isolated from adrenal glands [2]. It showed a potent inhibitory activity against 11β-hydroxysteroid dehydrogenase, which plays an important role in inflammation, salt retention, hypertension, obesity, diabetes, and occular hypertension [3]. Biotransformation of 11-ketoprogesterone (**1**) with various microorganisms and plant cell culture has been reported previously [4-7].

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In continuation of the studies on biotransformation of bioactive compounds [8-11], fermentation of **1** by various fungal strains, resulted in synthesis of transformed analogues **2-11**. These metabolites were unambiguously identified as pregna-1,4-diene-3,11,20-trione (**2**), androsta-1,4-diene-3,11,17-trione (**3**), 17$\beta$-hydroxy-androsta-1,4-diene-3,11-dione (**4**), 15$\alpha$,17$\beta$-dihydroxy-androsta-1,4-diene-3,11-dione-17-acetate (**5**), 15$\alpha$-hydroxy-pregna-1,4-diene-3,11,20-trione (**6**), 6$\beta$,15$\alpha$-dihydroxy-pregna-1,4-diene-3,11,20-trione (**7**), 15$\alpha$,17$\beta$-dihydroxy-androsta-1,4-diene-3,11-dione (**8**), 16$\alpha$,17$\beta$-dihydroxy-androsta-1,4-diene-3,11-dione (**9**), 15$\alpha$, 20*R*-dihydroxy-pregna-1,4-diene-3,11-dione (**10**), 15$\alpha$, 20*S*-dihydroxy-pregna-1,4-diene-3,11-dione (**11**). These include three known (**2-4)** and seven new polar metabolites (**5-11**). The structures of these metabolites were determined by spectroscopic studies and comparison with the spectral data of the substrate **1** and related other compounds. It was observed that *Fusarium lini* was one of the most efficient microorganisms to metabolize compound **1**.

## 2. Experimental

### 2.1. *Chemicals and instruments*

11-Ketoprogesterone (**1**) was purchased from MP Biomedicals. Silica gel precoated plates (Merck, $PF_{254}$; 20 × 20, 0.25 mm) were used for TLC based purification. Silica gel (70-230 mesh, Merck) was used for column chromatography. Melting points were determined with a Buchi-535 apparatus and were uncorrected. Optical rotations were measured in methanol with a JASCO DIP-360 digital polarimeter. UV Spectra (in nm) were recorded in methanol with a Hitachi U-3200 spectrophotometer. Infrared (IR) spectra (in $cm^{-1}$) were recorded in chloroform with an FT-IR-8900 spectrophotometer. The EI-MS were measured on a double focusing mass spectrometer (Varian MAT 312) while HREI-MS were recorded on Jeol JMS-HX 110 mass spectrometer. The $^{1}$H-NMR spectra were recorded in $CDCl_3$ on a Bruker Avance-300 MHz, AM-400 MHz and AMX-500 MHz instruments, while $^{13}$C-NMR spectra were recorded on a Bruker Avance-300 MHz, AM-400 MHz and AMX-500 MHz, operating at 75, 100 and 125 MHz, respectively, with tetramethylsilane as the internal standard. Methyl, methylene and methine carbons were distinguished by DEPT 90° and 135° experiments. Homonuclear $^{1}$H -$^{1}$H connectivities were deduced by COSY-45° experiment. One-bond $^{1}$H-$^{13}$C connectivities were determined by HMQC gradient pulse factor selection. Two- and three-bond $^{1}$H-$^{13}$C connectivities were determined by HMBC experiment. The chemical shifts ($\delta$ values) are reported in parts per million, relative to TMS at 0 ppm. The coupling constants (*J* values) are reported in Hertz.

### 2.2. *Microorganisms and culture medium*

The microbial cultures were originally obtained from the Northern Regional Research Laboratories (NRRL). Fungal cultures of *Fusarium lini* (NRRL 2204) was grown on Sabouraud 4% dextrose-agar (Merck) at 25 °C and stored at 4 °C. The medium for *F. lini*

was prepared by mixing the following ingredients into distilled $H_2O$ (4.0 L): glucose (40.0 g), glycerol (40.0 mL), peptone (20.0 g), yeast extract (20.0 g), $KH_2PO_4$ (20.0 g), and NaCl (20.0 g).

### 2.3. *Fermentation of compound 1 and extraction of metabolites*

100 mL of the fungal medium was transferred into each of forty 250 mL conical flasks and autoclaved at 121 °C. Mycelia of *F. lini* were transferred to all the flasks and incubated at 25 °C for two days on rotary shaker (128 rpm). After two days, compound **1** (800 mg), dissolved in acetone (30 mL), was evenly distributed among the 40 flasks and the flasks were placed on a rotary shaker (128 rpm) at 26 °C and fermentation was continued for further 14 days. In all experiments, one control flask without biomass (for checking substrate stability) and one flask without exogenous substrate (for checking endogenous metabolite) were used. After 14 days, the culture medium was filtered and extracted with chloroform (12 L) in three portions. The extract was dried over anhydrous $Na_2SO_4$, evaporated under reduced pressure and the resulting brown gum (2.0 g) was analyzed by thin-layer chromatography (silica gel).

### 2.4. *Isolation and identification of transformed products*

The crude gum was dissolved in methanol, absorbed on silica gel (2 g) and subjected to column chromatography. The eluent system consisted of gradient mixtures of petroleum ether and chloroform, followed by methanol.

2.4.1. *Pregna-1,4-diene-3,11,20- trione* (**2**)

White crystalline powder, mp: 179-181 °C, $[\alpha]^{25}_D$ = + 217 (*c* = 0.058, MeOH). UV (MeOH): $\lambda_{max}$ (log $\varepsilon$), 239 nm (4.07).

*2.4.2. Androsta-1, 4-diene-3, 11,17-trione* (**3**)

White crystalline solid, mp: 197-198 °C, $[\alpha]^{25}_D$ = + 253 (*c* = 0.218, MeOH). UV (MeOH): $\lambda_{max}$ (log $\varepsilon$), 238 nm (3.80).

2.4.3. *17α-Hydroxy-androsta-1, 4-diene-3, 11-dione* (**4**)

Crystalline solid, mp: 224-226 °C, $[\alpha]^{25}_D$ = + 170 (*c* = 0.134, MeOH). UV (MeOH): $\lambda_{max}$ (log $\varepsilon$), 237.2 nm (4.33).

2.4.4. *15α, 17β-Dihydroxy-androsta-1, 4-diene-3, 11-dione-17-acetate* (**5**)

White solid, mp: 210-212 °C, $[\alpha]^{25}_D$ = + 143 (*c* = 0.056, MeOH). UV (MeOH): $\lambda_{max}$ (log $\varepsilon$), 240 nm (4.05). IR ($CHCl_3$): 3405, 2922, 1733, 1709, 1661, 1609, 1241, 756 $cm^{-1}$.

$^{1}$H- and $^{13}$C- NMR ($CDCl_3$, 400 and 125 MHz, respectively): Tables- 1 and -2. EI-MS: *m/z* 358 (25, $M^+$), 330 (7), 298 (9), 280 (15), 252 (8), 238 (15), 236 (8), 223 (5), 201 (8), 213 (3), 187 (5), 173 (13), 161 (29), 160 (100), 147 (22), 134 (19), 132 (45), 121 (88), 117 (32), 105 (20), 95 (17), 91 (61), 77 (27), 55 (29). HREI-MS: *m/z* 358.1722 ($M^+$, $[C_{21}H_{26}O_5]^+$; calcd. 358.1780).

2.4.5. *15α-Hydroxy-pregna-1,4-diene-3,11,20-trione* (**6**)

White solid, mp: 187-189 °C, $[\alpha]^{25}_D$ = + 239 (*c* = 0.056, MeOH). UV (MeOH): $\lambda_{max}$ (log $\varepsilon$), 240 nm (4.21). IR ($CHCl_3$): 3433, 2946, 1705, 1660, 1617, 755 cm$^{-1}$. $^{1}$H- and $^{13}$C-NMR ($CDCl_3$, 500 and 125 MHz, respectively): Tables-1 and -2. EI-MS: *m/z* 342 (58, $M^+$), 314 (9), 280 (22), 237 (11), 199 (7), 187 (6), 185 (7), 172 (11), 171 (10), 161 (45), 160 (90), 147 (29), 145 (30), 132 (43), 121 (100), 117 (32), 105 (20), 95 (20), 91 (68), 77 (29), 71 (26), 55 (38). HREI-MS: *m/z* 342.1861 ($M^+$, $[C_{21}H_{26}O_4]^+$; calcd. 342.1831).

2.4.6. *6β,15α-Dihydroxy-pregna-1,4-diene-3,11,20-trione* (**7**)

White solid, mp: 194-195 °C, $[\alpha]^{25}_D$ = + 143 (*c* = 0.056, MeOH). UV (MeOH): $\lambda_{max}$ (log $\varepsilon$), 240 nm (4.21). IR ($CHCl_3$): 3403, 2925, 1703, 1660, 1615, 755 cm$^{-1}$. $^{1}$H- and $^{13}$C-NMR ($CDCl_3$, 400 and 125 MHz, respectively): Tables-1 and -2. EI-MS: *m/z* 358 (34, $M^+$), 322 (7), 314 (17), 280 (12), 278 (14), 213 (11), 176 (14), 171 (12), 161 (18), 159 (23), 149 (28), 147 (51), 135 (35), 131 (36), 121 (65), 117 (22), 109 (40), 107 (40), 91 (77), 77 (60), 71 (55), 55 (100). HREI-MS: *m/z* 358.1758 ($M^+$, $[C_{21}H_{26}O_5]^+$; calcd. 358.1780).

2.4.7. *15α, 17β-Dihydroxy-androsta-1, 4-diene-3, 11-dione* (**8**)

Colorless crystalline solid, mp: 226-227 °C, $[\alpha]^{25}_D$ = + 161 (*c* = 0.274, MeOH). UV (MeOH): $\lambda_{max}$ (log $\varepsilon$) 240 nm (4.43). IR ($CHCl_3$):3399, 2941, 1705, 1658, 1610, 753cm$^{-1}$. $^{1}$H- and $^{13}$C- NMR ($CDCl_3$, 500 and 125 MHz, respectively): Tables-1 and -2. EI-MS: *m/z* 316 (19, $M^+$), 282 (10), 264 (12), 238 (20), 225 (7), 213 (5), 199 (11), 184 (10), 173 (15), 161 (41), 160 (100), 147 (32), 145 (33), 132 (44), 131 (35), 121 (82), 117 (32), 107 (21), 91 (49), 77 (23), 71 (12), 55 (39). HREI-MS: *m/z* 316.1657 ($M^+$, $[C_{19}H_{24}O_4]^+$; calcd. 316.1675).

2.4.8. *16α, 17β-Dihydroxy-androsta-1, 4-diene-3, 11-dione* (**9**)

White crystalline solid, mp: 220-223 °C, $[\alpha]^{25}_D$ = + 168 (*c* = 0.056, MeOH). UV (MeOH): $\lambda_{max}$ (log $\varepsilon$), 239 nm (4.14). IR ($CHCl_3$): 3377, 2930, 1705, 1659, 1610, 755 cm$^{-1}$. $^{1}$H- and $^{13}$C- NMR ($CDCl_3$, 500 and 125 MHz, respectively): Table 1. EI-MS: *m/z* 316 (40, $M^+$), 288 (13), 272 (20), 212 (10), 186 (21), 173 (11), 171 (12), 161 (21), 160 (39), 159 (39), 147 (33), 145 (25), 131 (42), 121 (91), 105 (26), 91 (100), 77 (41), 71 (22), 55 (62). HREI-MS: *m/z* 316.1679 ($M^+$, $[C_{19}H_{24}O_4]^+$; calcd. 316.1675).

2.4.9. *15α, 20R-Dihydroxy-pregna-1,4-diene-3,11-dione* (**10**)

Colorless gum, $[\alpha]^{25}_D$ = + 294 (*c* = 0.034, MeOH). UV (MeOH): $\lambda_{max}$ (log $\varepsilon$), 240 nm (4.01). IR ($CHCl_3$): 3405, 2965, 1703, 1659, 1614, 755 $cm^{-1}$. $^1H$- and $^{13}C$- NMR ($CDCl_3$, 400 and 125 MHz, respectively): Tables-1 and -2. EI-MS: *m/z* 344 (30, $M^+$), 326 (5), 308 (2), 282 (4), 164 (8), 238 (15), 199 (11), 173 (17), 171 (27), 161 (42), 160 (100), 159 (40), 147 (26), 145 (78), 132 (62), 121 (65), 117 (30), 105 (26), 95 (17), 91 (44), 77 (22), 55 (46). 77 (22), 55 (71). HREI-MS: *m/z* 344.1975 ($M^+$, $[C_{21}H_{28}O_4]^+$; calcd. 344.1988).

2.4.10. *15α, 20S-Dihydroxy-pregna-1,4-diene-3,11-dione* (**11**)

Colorless gum, $[\alpha]^{25}_D$ = + 122 (*c* = 0.082, MeOH). UV (MeOH): $\lambda_{max}$ (log $\varepsilon$) 239 nm (4.14). IR ($CHCl_3$): 3406, 2966, 1705, 1659, 1611, 754 $cm^{-1}$. $^1H$- and $^{13}C$- NMR ($CDCl_3$ , 500 and 125 MHz, respectively): Tables-1 and -2. EI-MS: *m/z* 344 (50, $M^+$), 326 (4), 316 (12), 282 (6), 264 (8), 238 (15), 199 (7), 173 (13), 161 (38), 160 (100), 159 (34), 147 (33), 145 (33), 132 (44), 121 (99), 117 (35), 105 (30), 95 (22), 91 (72), 79 (31), 77 (22), 55 (71). HREI-MS: *m/z* 344.1971 ($M^+$, $[C_{21}H_{28}O_4]^+$; calcd. 344.1988).

Table 1. $^1H$-NMR data of new compounds 5-11 ($CDCl_3$).

| Position | **5** [a] | **6** [b] | **7** [a] | **8** [b] | **9** [b] | **10** [a] | **11** [b] |
|---|---|---|---|---|---|---|---|
| 1 | 7.63 | 7.71 | 7.65 | 7.65 | 7.66 | 7.64 | 7.67 |
| 2 | 6.20 | 6.20 | 6.20 | 6.20 | 6.19 | 6.19 | 6.19 |
| 3 | - | - | - | - | - | - | - |
| 4 | 6.06 | 6.16 | 6.07 | 6.07 | 6.06 | 6.07 | 6.06 |
| 5 | - | - | - | - | | - | - |
| 6 | 1.50 | 4.56 | 1.38 | 1.22 | 1.36 | 1.36 | 1.38 |
| | 2.22 | | 2.38 | 2.05 | 2.35 | 2.45 | 2.37 |
| 7 | 2.21 | 2.53 | 2.46 | 2.23 | 2.23 | 2.27 | 2.32 |
| | 2.42 | 2.65 | 2.62 | 2.42 | 2.41 | 2.43 | 2.76 |
| 8 | 2.22 | 2.66 | 2.17 | 2.03 | 2.12 | 2.16 | 2.17 |
| 9 | 1.98 | 2.00 | 2.00 | 1.95 | 1.95 | 1.97 | 1.97 |
| 10 | - | - | - | - | - | - | - |
| 11 | - | - | - | - | - | - | - |
| 12 | 2.32 | 1.61 | 2.36 | 2.36 | 2.36 | 2.32 | 2.38 |
| | 2.45 | 2.53 | 2.52 | 2.48 | 2.49 | 2.48 | 2.46 |
| 13 | - | - | - | - | - | - | - |
| 14 | 1.68 | 1.80 | 1.71 | 1.95 | 1.64 | 1.67 | 1.66 |
| 15 | 4.19 | 4.23 | 4.17 | 1.70 | 4.16 | 4.12 | 4.09 |
| | | | | 1.93 | | | |
| 16 | 2.19 | 1.74 | 1.76 | 4.18 | 2.07 | 1.86 | 1.67 |
| | 2.32 | 2.79 | 2.80 | | 2.16 | 2.34 | 2.90 |
| 17 | 4.57 | 2.63 | 2.58 | 3.69 | 4.55 | 1.55 | 1.67 |
| 18 | 1.44 | 1.64 | 1.43 | 1.43 | 1.44 | 1.44 | 1.44 |
| 19 | 0.81 | 0.71 | 0.67 | 0.78 | 0.77 | 0.73 | 0.79 |
| 20 | - | - | - | - | - | 3.68 | 3.66 |
| 21 | 2.03 | 2.10 | 2.09 | - | - | 1.19 | 1.14 |

[a]400 MHz, [b]500 MHz.

Table 2. $^{13}$C-NMR data of compounds 5-11 ($CDCl_3$) in 125 MHz.

| Position | **5** | **6** | **7** | **8** | **9** | **10** | **11** |
|---|---|---|---|---|---|---|---|
| 1 | 154.6 | 154.7 | 156.1 | 154.9 | 154.8 | 155.1 | 154.9 |
| 2 | 127.9 | 127.8 | 127.1 | 127.8 | 127.1 | 127.7 | 127.8 |
| 3 | 186.8 | 186.2 | 186.4 | 186.3 | 186.4 | 186.3 | 186.3 |
| 4 | 124.7 | 124.7 | 126.7 | 124.6 | 124.9 | 124.5 | 124.6 |
| 5 | 165.8 | 166.0 | 163.0 | 166.3 | 166.1 | 166.4 | 166.3 |
| 6 | 28.2 | 28.2 | 73.2 | 28.2 | 28.1 | 28.3 | 28.3 |
| 7 | 33.6 | 33.6 | 40.2 | 33.7 | 33.0 | 33.8 | 33.6 |
| 8 | 36.4 | 36.1 | 30.7 | 36.7 | 37.0 | 36.2 | 36.0 |
| 9 | 60.5 | 60.7 | 60.4 | 60.7 | 60.8 | 60.6 | 60.7 |
| 10 | 42.3 | 42.3 | 42.5 | 42.4 | 42.4 | 42.4 | 42.3 |
| 11 | 207.4 | 207.2 | 207.0 | 207.8 | 207.4 | 209.1 | 208.4 |
| 12 | 54.6 | 56.2 | 56.3 | 54.3 | 54.3 | 57.5 | 56.5 |
| 13 | 51.0 | 51.0 | 51.9 | 51.8 | 51.4 | 51.7 | 51.1 |
| 14 | 57.2 | 61.6 | 61.3 | 57.5 | 57.5 | 56.5 | 51.7 |
| 15 | 71.3 | 72.2 | 72.2 | 71.3 | 33.5 | 72.8 | 72.5 |
| 16 | 40.4 | 36.1 | 36.2 | 43.3 | 78.2 | 38.6 | 37.9 |
| 17 | 77.6 | 59.6 | 59.6 | 77.1 | 77.6 | 59.7 | 59.8 |
| 18 | 14.6 | 15.6 | 15.7 | 13.5 | 13.0 | 14.6 | 15.1 |
| 19 | 18.9 | 18.8 | 20.4 | 18.9 | 18.9 | 18.9 | 18.8 |
| 20 | 170.6 | 206.6 | 206.7 | - | - | 69.4 | 68.2 |
| 21 | 20.9 | 31.2 | 31.2 | - | - | 23.8 | 23.8 |

## 3. Results and Discussion

11-Ketoprogesterone (**1**) was efficiently transformed by *F. lini* in fourteen days. This yielded ten oxidative metabolites, **2- 11** (Fig. 1). Metabolites **2-4** were found to be known compounds [12-13], while **3** and **4** were early reported as biotransformed products of adrenosterone [13]. Compounds **5**-**11** were found to be new metabolites.

Compound **5** was obtained as a white solid. The HREI-MS exhibited an $M^+$ at *m*/*z* 358.1722 ($C_{21}H_{26}O_5$, calcd. 358.1780). The UV showed $\lambda_{max}$ at 240 nm, indicating the presence of a conjugated carbonyl system. The IR spectrum ($CHCl_3$) showed absorptions for OH (3405 $cm^{-1}$) and ester carbonyl (1733 $cm^{-1}$) groups. The $^1$H NMR spectrum showed two additional olefinic protons at $\delta$ 7.63 (d, $J$ = 10.2 Hz) and 6.20 (dd, $J$ = 10.2 Hz, $J$ = 1.6 Hz), along with a broad singlet at $\delta$ 6.06, and hydroxyl-bearing methine

protons at $\delta$ 4.97 (t, $J$ = 8.9 Hz) and 4.19 (m), as compared to **1** [4]. The $^{13}$C-NMR spectrum (broad-band decoupled and DEPT) showed signals for three olefinic methine carbons at $\delta$ 154.6, 127.9, and 124.7, two oxygen-bearing methine carbons at $\delta$ 77.6 and 71.3, and a quaternary carbon at $\delta$ 170.6. The olefinic methine signals at C-1 ($\delta$ 154.6), and C-2 ($\delta$ 127.9) were identified on the basis of HMBC correlations of Me-19 ($\delta$ 1.44) with C-1 ($\delta$ 154.6) and COSY 45° interactions between H-1 ($\delta$ 7.63) and H-2 ($\delta$ 6.20). The downfield methine carbon signal at $\delta$ 77.6 was assigned to C-17 on the basis of HMBC of H-17 ($\delta$ 4.97) with C-18 ($\delta$ 14.6) and C-12 ($\delta$ 54.6). Moreover the hydroxyl-bearing methine proton at $\delta$ 4.19 was assigned to H-15 on the basis of its COSY 45° correlations with H-14 ($\delta$ 1.68) and H-16 ($\delta$ 2.19). The quaternary carbon signal ($\delta$ 170.6) was assigned to C-20 ester carbonyl on the basis of its HMBC correlations with H-17 ($\delta$ 4.97) and Me-21 ($\delta$ 2.03). The stereochemistry at C-17 and C-15 were deduced on the basis of NOESY correlations of H-17 ($\delta$ 4.97) with H-14 ($\delta$ 1.68) and of H-15 ($\delta$ 4.19) with Me-18 ($\delta$ 0.81). From these data, the structure of metabolite **5** was deduced as 15$\alpha$,17$\beta$-dihydroxy-androsta-1,4-diene-3,11-dione-17-acetate. Compound **5** seems to be the result of enzyme catalyzed regio- and sterospecific Baeyer-Villiger oxidation of substrate **1** [4, 14].

Compound **6** exhibited the $M^+$ at $m/z$ 342.1861 ($C_{21}H_{26}O_4$, calcd. 342.1831), IR absorptions for hydroxyl, ketonic and alkene moieties at 3433, 1705 and 1617 cm$^{-1}$, respectively, $\lambda_{max}$ of conjugated ketone, at 240 nm and $^1$H NMR spectrum (similar to **1)** except two additional olefinic protons at $\delta$ 7.65 (d, $J$ = 10.2 Hz), and 6.20 (dd, $J$ = 10.2 Hz, $J$ = 1.2 Hz), along with hydroxyl bearing methine proton at $\delta$ 4.17 (m). The olefinic protons were assigned to H-1 and H-2 by comparison with **5**. The downfield methine carbon ($\delta$ 72.2) was assigned to C-15 on the basis of HMBC and COSY 45° correlations. The stereochemistry of C-15 OH was assigned to be $\alpha$ on the basis of NOESY correlations between H-15$\beta$ ($\delta$ 4.17) and Me-18 ($\delta$ 0.67). From this data, the structure of **6** was deduced as 15$\alpha$-hydroxy-pregna-1,4-diene-3,11,20-trione.

Compound **7** exhibited a $M^+$ at $m/z$ 358.1758 ($C_{21}H_{26}O_5$, calcd. 358.1780), IR absorptions for OH (3403 cm$^{-1}$), CO (1703 cm$^{-1}$) and C=C (1615 cm$^{-1}$), a conjugated enone system ($\lambda_{max}$ at 240 nm). The $^1$H NMR spectrum of **7** closely resembled to that of **6** with an additional signal at $\delta$ 4.56, which was assigned to H-6, geminal to an OH. The stereochemistry of C-6 OH was assigned to be $\beta$ on the basis of NOESY correlations between H-6 ($\delta$ 4.56) and H-9 ($\delta$ 2.00). From these data, the structure of metabolite **7** was deduced as 6$\beta$,15$\alpha$-dihydroxy-pregna-1,4-diene-3,11,20-trione.

Compound **8** was obtained as colorless crystals. The HREI-MS exhibited an $M^+$ at $m/z$ 316.1657 ($C_{19}H_{24}O_4$, calcd. 316.1675), absorptions for hydroxyl, ketonic and alkene moieties at 3399, 1705 and 1610 cm$^{-1}$, respectively and $\lambda_{max}$ at 240 nm. The absence of NMR signals corresponding to the C-17 acetyl group indicated that **8** was a deacetylated derivative of compound **5**. The C-17 ($\delta$ 77.1) was shifted downfields, while C-16 ($\delta$ 43.3) and C-13 ($\delta$ 47.8) shifted upfields, as compared to compound **5.** Thus **8** was identified as 15$\alpha$,17$\beta$-dihydroxy-androsta-1,4-diene-3,11-dione.

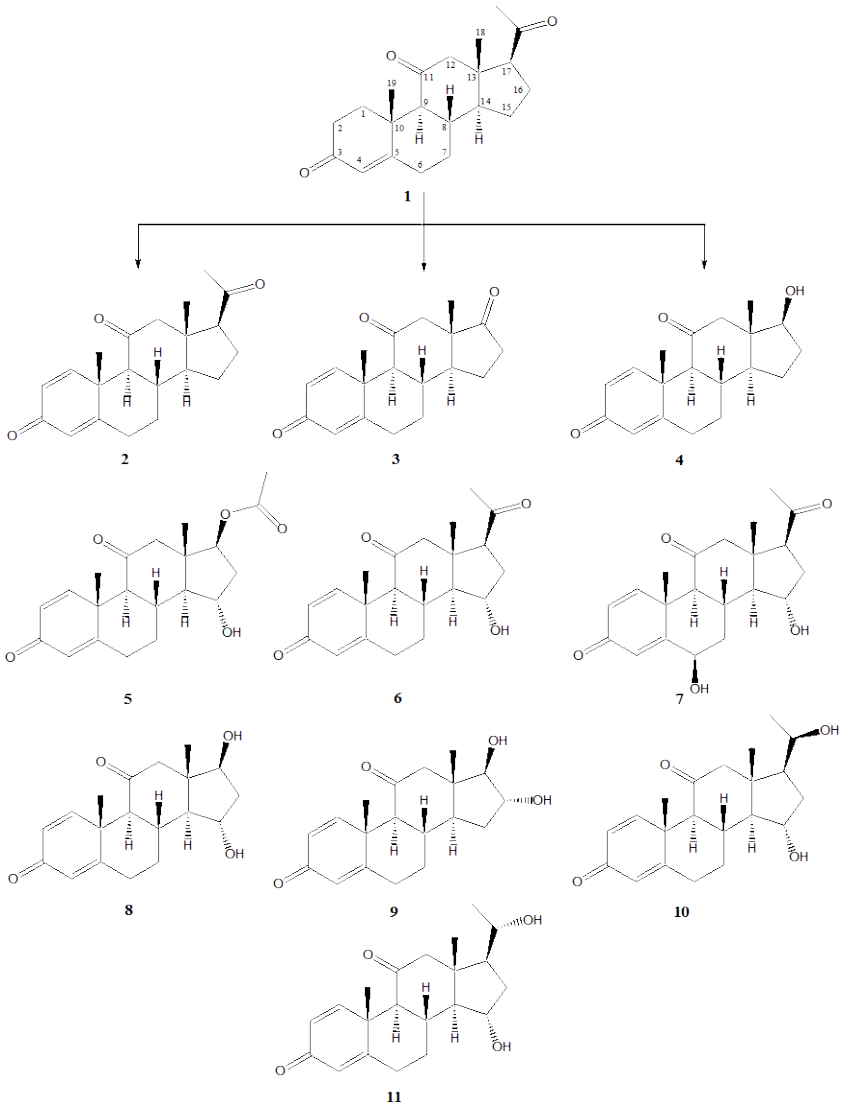

Fig. 1. Structures of substrate and biotransformed products by *Fusarium lini.*

Compound **9** showed $M^+$ at *m/z* 316.1679 ($C_{19}H_{24}O_4$, calcd. 316.1675), absorptions for OH (3377 $cm^{-1}$), CO (1710 $cm^{-1}$) and C=C (1610 $cm^{-1}$) and $\lambda_{max}$ of conjugated ketone (at 239 nm). The $^1H$ and $^{13}C$ NMR spectra of **9** were distinctly similar to those of **8**, except the chemical shifts of hydroxyl-bearing methine groups. The stereochemistry at C-17 and C-16 were deduced by NOESY correlations of H-17 ($\delta$ 3.69) with H-14 ($\delta$ 1.95) and of H-16 ($\delta$ 4.18) with Me-18 ($\delta$ 0.78). Based on the above analysis, the structure of metabolite **9** was deduced to be 16$\alpha$,17$\beta$-dihydroxy-androsta-1,4-diene-3,11-dione.

Compound **10** showed $M^+$ at *m/z* 344.1975 ($C_{21}H_{28}O_4$, calcd 344.1988), OH absorption at 3405 $cm^{-1}$. The $^1H$ and $^{13}C$ NMR spectra of **10** were distinctly similar to **1**, except additional signals at $\delta$ 4.09 (br. s) and 3.66 (br. s), and the absence of C-20 carbonyl signal ($\delta$ 207.6). The upfield shift of the methyl C-21 signal by about 7 ppm suggested the reduction of the vicinal C-20 ketonic carbonyl to hydroxyl functionality. This was deduced by the HMBC correlations. C-20 was assigned to be *R* [15]. The structure of metabolite **10** was thus identified to be 15$\alpha$, 20*R*-dihydroxy-pregna-1,4-diene-3,11-dione.

Compound **11** exhibited an $M^+$ at *m/z* 344.1971 ($C_{21}H_{28}O_4$, calcd. 344.1988) and an absorption at 3406 $cm^{-1}$ (OH). The $^1H$ and $^{13}C$ NMR spectra of **11** were distinctly similar to **10**, except the chemical shift of the C-18 methyl signal at $\delta$ 0.73, which suggested an *S* configuration at the C-20 of metabolite **11.** Based on the above data, the structure of metabolite **11** was deduced as 15$\alpha$, 20*S*-dihydroxy-pregna-1,4-diene-3,11-dione, an epimer of **10**.

In conclusion, incubation of 11-ketoprogesterone (**1**) with filamentous fungi *F. lini* yielded various oxidative metabolites, resulting from $\Delta^{1,2}$ dehydrogenation, hydroxylation, reduction, oxidative cleavage of side chain and Baeyer-Villiger oxidation of **1**. Reduction of C-20 carbonyl group occurred, followed by cleavage of side chain to form androstane derivatives. In the case of reduction of C-20 carbonyl group, it was found to be non-stereospecific, giving rise to both *R* and *S* isomers. However interestingly no reduction of C-11 ketonic carbonyl group was observed. These transformation showed high stereo-selectivity and are therefore difficult to synthesize by general chemical means. This work therefore demonstrates the tremendous mechanistic advantages of microbial transformations, as compared to conventional chemical conversion.

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