# Microbial conversion of progesterone with *Aspergillus* sp.

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**Abstract.** The conversion of progesterone (1) by *Aspergillus* sp. was studied. The major metabolites formed from progesterone were identified as 11α-hydroxyl progesterone (2), 15α-hydroxyl progesterone (3), 15β-hydroxypreg-1,4-dien-3,20-dione (4), 6β,11α-dihydroxypregesterone (5).Conversion products were characterized by spectroscopic methods including $^{1}$H NMR, $^{13}$C NMR and MS.

## Introduction

Microbial conversion of steroids provide an important method of obtaining new steroid derivatives of potential pharmaceutical activity, which additionally fulfills green chemistry principles[1-2]. The major advantage of biological catalysts is their capability to catalyze novel reactions with regio- and stereo-selectivity[3]. Since 11α- hydroxylation of progesterone by *Rhizopus arrhizus* was patented in 1952[4]. A wide variety of microorganisms have been investigated and have been successfully applied to transform a wide range of steroids to produce functionalized compounds with therapeutic use and commercial value[5-7].

The *Aspergillus* genus is an important source of fungal pathogen which can cause severe seed yield loss in soybeans and other crops[8]. However, strains of *Aspergillus* genus had never used in the biotransformation of steroids. During our screening program for isolation of microorganism from *Paris polyphylla* Smith var. Yunnanenis capable of transforming progesterone, a strain of *Aspergillus* sp. was isolated from rhizome of *Paris polyphylla* Smith var. yunnanenis and applied for converting progesterone hydroxylation in different position.

## Materials and methods

### Chemicals

Progesterone (1) was purchased from Yancheng Xinyi Pharm&Che Co., Ltd, Yancheng, China. It was assayed at >99% purity by HPLC (PDA as detector).

### Microorganism

The fungal strain *Aspergillus* sp. was isolated from *Paris polyphylla* Smith var. yunnanenis collected from Baoshan, Yunnan province, PRC. The strain of *Aspergillus* sp. was grown at 28C and stored at4C on agar slopes composed of Glucose (30.0 g), potato (100.0 g),agar (20 g) mixed into distilled $H_2O$ (1 L).

### Culture conditions and transformation

*Aspergillus* sp. broth media were transferred into 250 mL conical flask (100 mL each). It was prepared with medium (1 L): Glucose (30.0 g), $K_2HPO_4$ (1 g), $MgSO_4 \cdot 7H_2O$ (0.5 g), KCl (0.5 g), $FeSO_4$(0.01 g), and pH was maintained at 5.8. 10 g progesterone and 4 g Tween80 dissolved in acetone were mixed into broth media (10 L).Seed flasks without progesterone were prepared from three-day old slants and allowed for one day on a shaker at 28C. The remaining flasks within progesterone were inoculated from the seed flasks and placed on a rotatory shaker (180 rpm) at 28C for fermentation for 96 h.

**Analysis**

Melting points were determined by a Buchi 535 melting-point apparatus. Optical rotations were measured on a Jasco P-2000 digital polarimeter. EI-MS was recorded on an Agilent 5973N. $^{1}H$ NMR and $^{13}C$ NMR spectra were performed on Bruker 400 spectrometers with TMS as internal standard. Column chromatography was performed on silica gel (200-300 mesh; Qingdao). Thin layer chromatography (TLC) analysis was carried out on precoated plates with 0.20-0.25 mm thick silica gel GF254 (Qingdao) with detection by heating silica gel plates sprayed with 10% $H_2SO_4$ in EtOH.

**Isolation of transformed products**

The mycelium was filtered from the fermentation broth and extracted three times with ethanol, while the broth was extracted three times with EtOAc. That was evaporated under reduced pressure and brown crude (10.53 g) was obtained. Then the crude was subject to silica gel CC to afford Compound 2 (345 mg), 3 (123 mg), 4 (13 mg), 5 (18 mg) on elution with petroleum ether/EtOAc (9:1-1:9), Chemical structures of the products were determined by means of spectral data and mentioned in respective order.

## Results and discussion

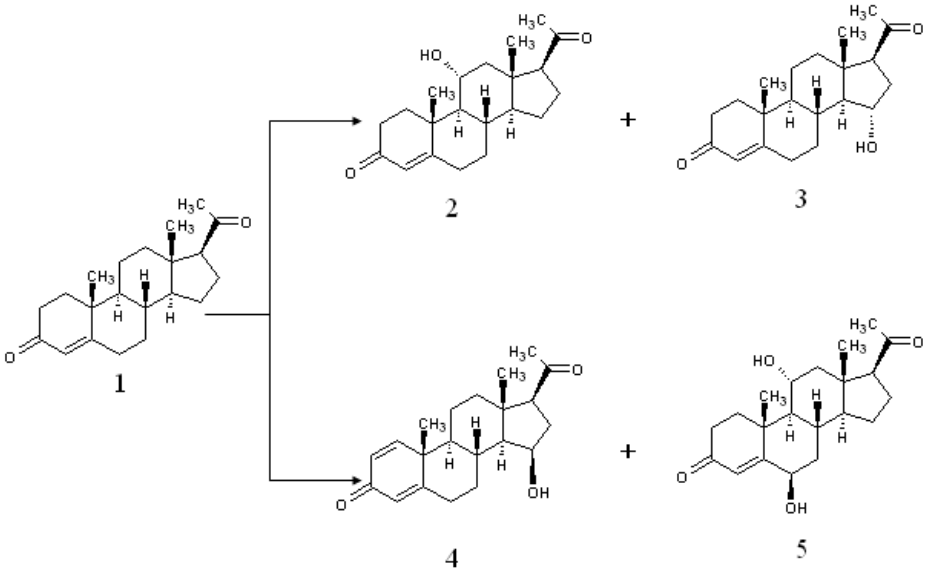


**Fig. 1.** The structures of Progesterone (1) and the biotransformed products 11α-hydroxyl progesterone (2), 15α-hydroxyl progesterone (3), 15β-hydroxypreg-1,4-dien-3,20-dione (4), 6β,11α–dihydroxypregesterone (5).

*A.versicolor* converted progesterone into the compound 2, 3, 4 and 5 (Fig. 1). All of them are more polar than the substrate itself. $^{13}CNMR$ data of the substrate as well as the conversion products are listed in Table 1. The analytical data of compound 2, 3, 4 and 5 are mentioned in respective order.

11α-hydroxyprogesterone (2) Crystallized from methanol; m.p.: 155-157ºC. $^{1}H$ NMR (CDC $l_3$, 400 MHz,δin ppm，multi.，J in Hz): 5.70(1H, m, H-4)，0.65 (3H, s, H-18), 1.2 (3H, s, H-19), 4.99 (1H, m, H-11). ESI-MS: m/z 354.2 $[M+Na]^+$; m/z 369.2 $[M+K]^+$. Mass spectru m indicated a molecular of composition $C_{21}H_{30}O_3$. It unequivocally confirms the structure o f 11α-hydroxyprogesterone[9,10].

15α-hydroxyl progesterone (3) Crystallized from methanol; m.p.: 229-231C (methanol). $^{1}H$ NMR ($CDCl_3$, 400 MHz,δ in ppm，multi., J in Hz): 5.64 (1H, m, H-4), 4.10 (1H, m, H-15β), 2.81 (1H, t, H-17α), 0.67 (3H, s, H-8), 1.17 (3H, s, H-19), 2.12 (3H, s, H-21).ESI-M S: m⁄z: 353 $[M+Na]^+$. Mass spectrum indicated a molecular of composition $C_{21}H_{30}O_3$. It is in accordance with the literature data for 15α-hydroxyl pregesterone[11].

15β-hydroxypreg-1,4-dien-3,20-dione (4) Crystallized from methanol; m.p.: 229-234ºC. $^{1}H$ NMR ($CDCl_3$, 400 MHz,δ in ppm, multi., J in Hz): 6.09 (1H, s, H-4), 7.06 (1H, d, H-1),

6.20 (1H, dd, H-2) ,2.42 (1H, m, H-17), 0.88 (3H, s,H-18), 1.20 (3H, s, H-19), 2.08 (3H, s, H-21).ESI-MS: m/z 351 $[M+Na]^+$. Mass spectrum indicated a molecular of composition $C_{21}H_{28}O_3$.It is in accordance with the literature data for 11α-hydroxypreg-1,4-dien-3,20-dione[12].

6β,11α-dihydroxypregesterone (5) Crystallized from methanol;m.p.: 245-248ºC. $^1H$ NMR ($CDCl_3$, 400 MHz, δ in ppm, multi., J in Hz): 5.78 (s, 1H, H-4 ), 2.68 (t, 1H, H-17), 0.73 (s, 3H, H-18), 1.49 (s, 3H, H-19), 2.14 (s, 3H, H-21),4.34 (m, 1H, H-6), 4.09 (m, 1H,H-11).ESI-MS: m/z 369 $[M+Na]^+$. Mass spectrum indicated a molecular of composition $C_{21}H_{30}O_4$. It is in accordance with the literature data for 6β,11α-dihydroxypregesterone[13].

**Table.1**. $^{13}C$ NMR signals of the substrate and metabolites (δ in ppm downfield from TMS, in $CDCl_3$)

| C | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| 1 | 35.7 | 37.6 | 35.8 | 156.8 | 37.5 |
| 2 | 34.0 | 34.3 | 34.1 | 127.1 | 34.1 |
| 3 | 199.4 | 200.3 | 199.6 | 187.0 | 202.7 |
| 4 | 123.9 | 124.7 | 124.0 | 123.4 | 126.0 |
| 5 | 170.9 | 170.9 | 170.8 | 170.6 | 170.5 |
| 6 | 32.8 | 33.7 | 32.8 | 32.6 | 72.1 |
| 7 | 31.9 | 31.6 | 32.1 | 32.5 | 38.6 |
| 8 | 35.6 | 35.1 | 35.3 | 31.6 | 28.2 |
| 9 | 53.7 | 59.1 | 53.8 | 52.5 | 58.4 |
| 10 | 38.7 | 40.1 | 38.7 | 43.8 | 39.2 |
| 11 | 21.0 | 69.0 | 21.0 | 22.7 | 68.0 |
| 12 | 38.5 | 50.6 | 38.9 | 39.9 | 49.4 |
| 13 | 43.9 | 44.26 | 44.7 | 43.9 | 44.1 |
| 14 | 56.0 | 55.5 | 62.9 | 59.7 | 55.1 |
| 15 | 24.4 | 24.4 | 73.5 | 69.6 | 23.9 |
| 16 | 22.8 | 23.1 | 35.4 | 35.7 | 22.7 |
| 17 | 63.5 | 63.2 | 61.0 | 63.5 | 63.1 |
| 18 | 13.3 | 14.6 | 14.8 | 15.7 | 14.1 |
| 19 | 17.4 | 18.4 | 17.6 | 18.4 | 19.5 |
| 20 | 209.3 | 208.9 | 208.4 | 209.3 | 210.6 |
| 21 | 31.5 | 31.5 | 31.7 | 31.2 | 31.0 |

## Conclusion

The conversion products of progesterone with Aspergillus sp. including 11α-hydroxyl progesterone (2), 15α-hydroxyl progesterone (3), 15β-hydroxypreg-1,4-dien-3,20-dione (4), 6β,11α-dihydroxypregesterone(5).The hydroxylation positions include 6β, 11α, 15α and 15β as well as a reaction of dehydrogenation between C1 and C2.

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