

Microbial transformation of dydrogesterone by *Gibberella fujikuroi*

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Abstract

Microbial transformation of dydrogesterone ($9\beta,10\alpha$ -pregna-4,6-diene-3,20-dione) (**1**) on fermentation with *Gibberella fujikuroi* yielded 20R-hydroxy- $9\beta,10\alpha$ -pregna-4,6-diene-3-one (**2**), 17 β -hydroxy- $9\beta,10\alpha$ -androsta-4,6-diene-3-one (**3**) and $9\beta,10\alpha$ -androsta-4,6-diene-3,17-dione (**4**). Their structures were deduced on the basis of modern spectroscopic techniques. This is the first report of their production by using *Gibberella fujikuroi*.

Keywords: Microbial transformation, biotransformation, dydrogesterone, *Gibberella fujikuroi*.

Introduction

Chemical compounds, synthesized or isolated from the natural sources, have been subjected to structural modifications to obtain desirable properties (Salemme et al. 1997). Microbial transformation is one of the important tools for structural changes. Thousands of microbial transformations involving different types of reactions including hydroxylation, oxidation, reduction, acetylation with organic compounds and natural products have now become known; some of them have proved to be very useful for synthetic organic chemistry.

Dydrogesterone (**1**) is a synthetic hormone similar to the naturally occurring sex hormone, progesterone (Choudhary et al. 2008). It is used to treat premenstrual syndrome, period pains, irregular periods, infertility and endometriosis, which are all due to the

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deficiency of progesterone. It may also be used to minimise the overgrowth of the womb lining due to oestrogen in hormone replacement therapy (HRT) following menopause. By maintaining the womb lining, dydrogesterone (**1**) is also useful in helping to prevent miscarriage in women who have suffered repeated miscarriages in the past.

In continuation of our biotransformational studies of bioactive organic compounds (Choudhary et al. 2008; Choudhary et al. 2002; Azizuddin et al. 2008; Azizuddin et al. 2010), fermentation of dydrogesterone (**1**) with *Gibberella fujikuroi* yielded compounds **2-4** (Table 1) which were reported for the first time from this fungal strain. The structures of compounds **2-4** were revealed with the aid of spectroscopic studies and corresponding data were reported. (Choudhary et al. 2008).

Table 1: Percentage yields of metabolites of dydrogesterone (**1**) by fermentation with *Gibberella fujikuroi* after 12 days.

Microorganism	Yield of 2 (%)	Yield of 3 (%)	Yield of 4 (%)
<i>Gibberella fujikuroi</i>	7.6	12.6	8.2

Materials and Methods

General methods

The $^1\text{H-NMR}$ spectrum was recorded in CDCl_3 on Bruker AM-400 NMR spectrometer with TMS as an internal standard using UNIX operating system at 400 MHz. The $^{13}\text{C-NMR}$ spectra were recorded in CDCl_3 at 100 MHz on Bruker AM-400 NMR spectrometer. The HREIMS was recorded on Jeol JMS 600 and HX 110 mass spectrometers with the data system DA 5000. The IR spectra were recorded on a Jasco A-302 spectrophotometer. The UV spectra were recorded on a Hitachi U-3200 spectrophotometer. The optical rotations were measured on JASCO DIP-360 digital polarimeter. The melting point was determined on a Buchi 510 apparatus. Column chromatography (CC) was carried on silica gel column (70-230 Mesh). Purity of the samples was checked by TLC on pre-coated silica gel GF-254 preparative plates (20 × 20 cm, 0.25 mm thick, Merck) and were detected under the UV light (254 and 366 nm), while ceric sulphate was used as spraying reagent. Dydrogesterone (**1**) was purified from the tablets of a medicinal

product, duphaston (10 mg each, manufactured by Solvay Pharmaceuticals, Belgium).

Table 2: ^1H NMR (400 MHz, CDCl_3)^a chemical shifts of dydrogesterone (1) and its metabolites 2-4. δ in ppm and J in Hz.

C. NO.	1	2	3	4
1	2.42 (m) 2.55 (m)	2.44 (m) 2.56 (m)	2.42 (m) 2.53 (m)	2.44 (m) 2.56 (m)
2	2.26 (m) 1.67 (m)	2.26 (m) 1.68 (m)	2.25 (m) 1.64 (m)	2.26 (m) 1.65 (m)
3	-	-	-	-
4	5.67 (br.s)	5.65 (br. s)	5.65 (br.s)	5.69 (s)
5	-	-	-	-
6	6.15 (d, $J=9.8$)	6.15 (d, $J=9.8$)	6.15 (d, $J=10.2$)	6.23 (d, $J=10.2$)
7	6.15 (d, $J=9.8$)	6.16 (d, $J=9.8$)	6.15 (d, $J=10.2$)	6.22 (d, $J=10.2$)
8 β	2.40 (m)	2.40 (m)	2.45 (m)	2.60 (m)
9 β	1.84 (m)	1.81 (m)	1.86 (m)	1.92 (m)
10	-	-	-	-
11	1.98 (m) 1.83 (m)	1.94 (m) 1.81 (m)	1.95 (m) 1.86 (m)	2.02 (m) 1.89 (m)
12	1.95 (m) 1.62 (m)	1.78 (m) 1.36 (m)	1.75 (m) 1.26 (m)	2.10 (m) 1.78 (m)
13	-	-	-	-
14 α	1.76 (m)	1.64 (m)	1.58 (m)	1.93 (m)
15	1.69 (m) 1.80 (m)	1.67 (m) 1.79 (m)	1.40 (m) 1.76 (m)	2.12 (m) 1.82 (m)
16	1.76 (m) 1.89 (m)	1.60 (m) 1.75 (m)	1.45 (m) 2.09 (m)	1.46 (m) 1.75 (m)
17 α	2.53 (m)	1.35 (m)	3.66 (t, $J=8.5$)	-
18	0.69 (s)	0.74 (s)	0.82 (s)	0.95 (s)
19	1.29 (s)	1.25 (s)	1.23 (s)	1.24 (s)
20	-	3.73 (quintet, $J=6.2$)	-	-
21	2.12 (s)	1.22 (d, $J=6.3$)	-	-

^a Assignments based on COSY and HMQC.

Preparation of fermentation media

Four liter media for *G. fujikuroi* (ATCC 10704) was prepared by mixing glucose (320 g), NH_4NO_3 (2 g), KH_2PO_4 (20 g), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (4 g), and Giberella trace element solution (8 mL) into distilled water (4 L). The fermentation medium thus obtained was distributed equally among 40 flasks of 250 mL capacity (100 mL in each) and autoclaved.

Cultivation of the microbes

Two-day-old spores of the four microbes were transferred into the broth medium flasks (250 mL) of their respective media containing freshly prepared and autoclaved media (100 mL). The seed flasks of the fungi were incubated on a shake table at 30°C for two days.

Inoculation of the cultures

Broth culture (100 mL) from 2-day-old seed flasks of the fungi were equally distributed to 38 media flasks (250 mL) containing the respective media (100 mL). The incubation was continued for a further 2 days for fungi.

Fermentation of dydrogesterone (1)

Dydrogesterone (1) (300 mg) dissolved in acetone (20 mL) and the resulting solution was evenly distributed among 38 conical flasks containing shake cultures of microbes, the fermentation was

continued for 12 days.

Extraction and isolation

Each mycelium was filtered, washed with EtOAc (400 mL) and the broth thus obtained was extracted with EtOAc (5 L). The ethyl extract was dried over anhydrous sodium sulfate and concentrated *in vacuo* to afford a brown gum (approximately, 1.24 g), which was adsorbed on flash silica gel (3 g), subjected to column chromatography. Elution with pet. ether: EtOAc (7.2:2.8), pet. ether: EtOAc (7.0:3.0) and pet. ether: EtOAc (7.7:2.3) afforded 2-4, respectively.

Table 3: ^{13}C NMR (100 MHz, CDCl_3)^b chemical shifts of dydrogesterone (1) and its metabolite 2-4.

C. NO.	1	2	3	4
1	34.0 (CH_2)	34.0 (CH_2)	34.0 (CH_2)	33.9 (CH_2)
2	35.6 (CH_2)	35.6 (CH_2)	35.5 (CH_2)	35.6 (CH_2)
3	199.3 (C)	199.5 (C)	199.5 (C)	199.3 (C)
4	123.9 (CH)	123.7 (CH)	123.8 (CH)	124.4 (CH)
5	162.9 (C)	163.3 (C)	163.2 (C)	162.4 (C)
6	127.1 (CH)	126.9 (CH)	127.0 (CH)	127.9 (CH)
7	140.3 (CH)	141.0 (CH)	140.3 (CH)	138.4 (CH)
8	38.6 (CH)	38.4 (CH)	38.7 (CH)	38.1 (CH)
9	39.7 (CH)	39.8 (CH)	40.1 (CH)	40.4 (CH)
10	37.2 (C)	37.2 (C)	37.2 (C)	37.2 (C)
11	20.6 (CH_2)	20.3 (CH_2)	20.2 (CH_2)	19.8 (CH_2)
12	37.8 (CH_2)	37.7 (CH_2)	35.6 (CH_2)	35.4 (CH_2)
13	44.2 (C)	42.0 (C)	43.2 (C)	47.7 (C)
14	49.9 (CH)	49.5 (CH)	44.7 (CH)	45.4 (CH)
15	22.6 (CH_2)	24.9 (CH_2)	24.2 (CH_2)	22.6 (CH_2)
16	25.2 (CH_2)	25.2 (CH_2)	30.1 (CH_2)	30.4 (CH_2)
17	63.4 (CH)	58.3 (CH)	81.6 (CH)	219.6 (C)
18	12.1 (CH_3)	11.3 (CH_3)	9.9 (CH_3)	12.3 (CH_3)
19	22.3 (CH_3)	22.2 (CH_3)	22.2 (CH_3)	22.2 (CH_3)
20	208.8 (C)	70.0 (CH)	-	-
21	31.4 (CH_3)	23.6 (CH_3)	-	-

^aMultiplicities were determined by DEPT experiments.

^bAssignment based on HMQC and HMBC.

20R-Hydroxy-9 β ,10 α -pregna-4,6-diene-3-one (2)

Colorless crystalline solid; m.p. 165-166 °C; $[\alpha]_{\text{D}}^{25}$ -118.7° (*c* 0.032, CHCl_3); UV (MeOH) λ_{max} (log ϵ): 286 nm (4.99); IR (CHCl_3) ν_{max} : 3408 (OH), 1725 (C=O), 1659, 1620 (C=C), 1228 cm^{-1} (C-O); FDMS m/z 314 [M^+ , 100 %]; EIMS m/z (rel. int. %): 314 [M^+] (08), 296 [$\text{M}^+-\text{H}_2\text{O}$] (100), 281 [$\text{M}^+-\text{H}_2\text{O}-\text{CH}_3$] (56), 267 (21), 227 (18), 201 (07), 161 (44), 135 (34), 105 (19), 83 (57); HREIMS m/z 314.2358 (M^+ , calcd 314.2352 for $\text{C}_{21}\text{H}_{30}\text{O}_2$); ^1H (CDCl_3 , 400 MHz) and ^{13}C NMR (CDCl_3 , 100 MHz) data listed in tables 2 and 3, respectively.

17 β -Hydroxy-9 β ,10 α -androsta-4,6-diene-3-one (3)

Colorless crystalline solid; m.p. 176 °C; $[\alpha]_{\text{D}}^{25}$ -63.6° (*c* 0.066, CHCl_3); UV (MeOH) λ_{max} (log ϵ): 286 nm (4.20); IR (CHCl_3) ν_{max} : 3417 (OH), 1727 (C=O), 1654, 1622 (C=C), 1233 cm^{-1} (C-O); FDMS m/z 286 [M^+ , 100 %]; EIMS m/z (rel. int. %): 286 [M^+] (100), 268 [$\text{M}^+-\text{H}_2\text{O}$] (30), 253 [$\text{M}^+-\text{H}_2\text{O}-\text{CH}_3$] (38), 214 (31), 199 (15), 172 (20), 133 (30), 107 (24), 83 (87); HREIMS m/z 286.1928 (M^+ , calcd 286.1930 for $\text{C}_{19}\text{H}_{26}\text{O}_2$); ^1H (CDCl_3 , 400 MHz) and ^{13}C NMR (CDCl_3 , 100 MHz) data listed in tables 2 and 3, respectively.

9 β ,10 α -Androsta-4,6-diene-3,17-dione (4)

Colorless solid; m.p. 196 °C; $[\alpha]_{\text{D}}^{25}$ +170° (*c* 0.02, CHCl_3); UV (MeOH) λ_{max} (log ϵ): 284 nm (4.18); IR (CHCl_3) ν_{max} : 1736 (C=O), 1659, 1623 cm^{-1} (C=C); EIMS m/z (rel. int. %): 284 [M^+] (37), 266

(12), 240 (12), 209 (7), 185 (5), 149 (7), 107 (11); HREIMS m/z 284.1813 (M^+ , calcd 284.1802 for $C_{18}H_{24}O_2$); 1H (CDCl₃, 400 MHz) and ^{13}C NMR (CDCl₃, 100 MHz) data listed in tables 2 and 3, respectively.

Results and Discussion

Microbial transformation of dydrogesterone (**1**) by using *Gibberella fujikuroi* yielded compounds **2-4** (Fig. 1). Their structures were found to be 20*R*-hydroxy-9 β ,10 α -pregna-4,6-diene-3-one (**2**) (Choudhary et al. 2008), 17 β -hydroxy-9 β ,10 α -androsta-4,6-diene-3-one (**3**) (Choudhary et al. 2008) and 9 β ,10 α -androsta-4,6-diene-3,17-dione (**4**) (Choudhary et al. 2008), which were identified with the aid of spectroscopic methods and compared with reported data. Such structures were reported for the first time from dydrogesterone by using *Gibberella fujikuroi*.

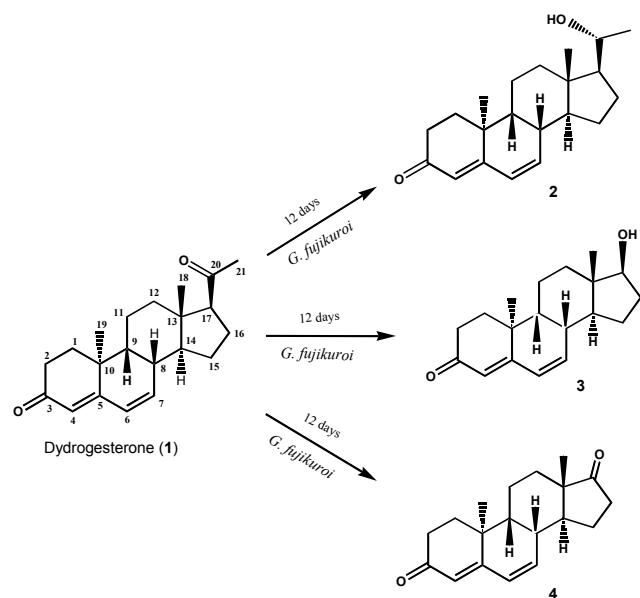


Figure 1. Microbial Transformation of Dydrogesterone (**1**) by *G. fujikuroi*.

Respiratory burst inhibitory activity of dydrogesterone (**1**) and its metabolites **2-4** have been compared with a previous study (Choudhary et al. 2008). Finally, in the present study, we attempted to investigate the biological activities of dydrogesterone (**1**) and its transformed products **2-4**, but it was rather difficult due to insufficient quantities.

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