Short Communication

Microbial Transformation of Nandrolone Decanoate by Acremonium Strictum

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Estr-4-en-3,17-dione **II**, 17β-hydroxyestr-4-en-3-one **III**, 15α-hydroxyestr-4-en-3,17-dione **IV**, and 15α,17β-dihydroxyestr-4-en-3-one **V** were produced by microbial transformation of nandrolone decanoate **I** in the culture of *Acremonium strictum* PTCC 5282. Bioconversion characteristics observed were ester hydrolysis, oxidation, and hydroxylation. Each microbial product was purified chromatographically and characterized on the basis of spectral data obtained from ^{1}H-NMR, ^{13}C-NMR, FT-IR, MS, and physical constants such as melting point and optical rotation.

Keywords: *Acremonium strictum* / Microbial transformation / Nandrolone decanoate / Steroid

Received: November 8, 2005; Accepted: December 9, 2005

DOI 10.1002/ardp.200500235

Introduction

Since the first comprehensive study by Mammoli and Vercelone, who described the involvement of microorganisms in steroid bioconversions, a series of reports have been published focusing on various aspects of microbial steroid transformations, some of them describing the production of pharmaceutical steroids. For example, Murray and Peterson patented 11α-hydroxylation of progesterone by *Rhizopus arrhizus*. Today, it is assumed that almost all steroids can be microbially hydroxylated [1]. Fungi are mainly involved in steroid bioconversion processes [2–6].

Acremonium strictum, a cephalosporium-like fungus, is able to hydroxylate different sites of the steroid backbone. Microbial addition of 1α-hydroxyl group using *A. strictum* was reported by Ambrus and coworkers [7]. Yoshihama and Nakakoshi reported steroid hydroxylations at various positions included 6β [6, 8, 9], 7α [6], 7β [10], 11α [6, 8, 9], 14α [6, 11], 15β [9, 10], 17α [6, 9], and 17β [10] with *A. strictum*. Our previous works on bioconversion of two pregnane-based steroids (hydrocortisone and progesterone) describe microbial modification of C-15 and side chain transformations by a strain of *A. strictum* isolated from soil [12, 13]. In the present study, we examined this strain for biotransformation of nandrolone decanoate to investigate the bioconversional effect of *A. strictum* PTCC 5282 on an estrane-like steroid.

Results and discussion

Biotransformation of 1 g nandrolone decanoate **I** by *Acremonium strictum* PTCC 5282 resulted in four steroid compounds **II-V**. During a cultivation period of six days (150 rpm at 25°C), the strain converted the substrate **I** into 204 mg of **II**, 166 mg of **III**, 49 mg of **IV**, and 56 mg of **V**, respectively. Microbial products were isolated and purified using chloroform extraction followed by preparative TLC with chloroform/acetone (8:2) and crystallization in methanol. Chemical structures of the products (Fig. 1)

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* This paper is dedicated to the memory of Dr. Seyedeh Maryam Zanjanian, deceased June 21, 2003.

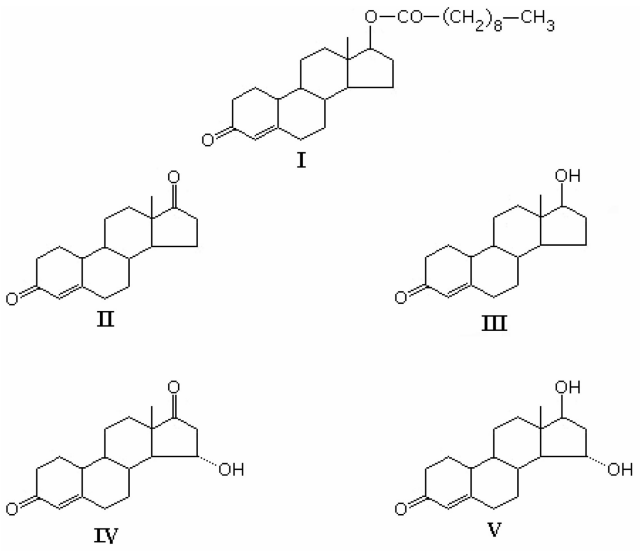

Figure 1. The structures of the substrate and biotransformed metabolites. Nandrolone decanoate **I**, estr-4-en-3,17-dione **II**, 17β-hydroxyestr-4-en-3-one **III**, 15α-hydroxyestr-4-en-3,17-dione **IV**, and 15α,17β-dihydroxyestr-4-en-3-one **V**.

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

Carbon atom	Compounds				
	I	II	III	IV	V
1	27.4	26.5	26.1	26.5	26.1
2	36.4	36.4	36.3	36.4	36.4
3	199.7	199.7	199.9	199.9	199.9
4	124.5	124.8	124.5	124.7	124.5
5	166.2	165.8	166.7	165.7	166.3
6	35.3	35.7	35.4	35.4	35.4
7	31.7	31.2	30.3	31.2	30.8
8	40.1	39.9	40.7	39.8	39.9
9	49.4	49.5	49.7	49.4	49.6
10	42.4	42.4	42.5	42.4	42.5
11	26.5	25.6	26.5	25.7	26.6
12	36.5	29.8	36.5	30.9	36.6
13	42.6	47.6	42.9	50.7	44.5
14	49.3	50.1	49.5	56.7	57.8
15	23.2	21.6	23.1	70.0	72.2
16	30.5	35.2	30.6	46.1	31.2
17	82.1	220.4	81.6	216.0	78.8
18	12.0	13.0	11.1	15.4	12.6

were determined by ^{1}H-NMR, ^{13}C-NMR, FT-IR, and MS. Additionally, physical characteristics such as melting point and optical rotation were determined. ^{13}C-NMR data of the substrate as well as the products are listed in Table 1.

Loss of side chain in compound **III** was approved according to the molecular ion at m/z 274 and change in the H-17 signal of the ^{1}H-NMR spectrum (from δ 4.5 in the substrate to δ 3.7). Chemical shift of δ 220.4 for 17-carbonyl group in ^{13}C-NMR, absence of H-17 signal in ^{1}H-NMR around δ 3.5–4.5, and the occurrence of the molecular ion at m/z 272 indicated oxidation of C-17 in compound **III** resulting in compound **II**. These two metabolites were identified as 17β-hydroxyestr-4-en-3-one and estr-4-en-3,17-dione, respectively, and have been previously reported by Hanson and coworkers [14].

Deshielding effect of 15α-hydroxyl group in 5α-androstanes on C-14, C-15, and C-16 were reported as δ +7.2, +50.2, and +12.4, respectively [15]. We have also shown these effects as δ +6.6, +49, and +12.9 in 5α-pregnanes [13]. Our observation was very similar to those found for **IV** (in comparison to **II)** suggesting the hydroxylation at C-15. As it is shown in Table 1, they occur as δ +6.6, +48.4, and +10.9, respectively.

The ^{13}C-NMR spectrum of compound **V** is similar to that of 15β,17β-dihydroxyestr-4-en-3-one [10] indicating probable α– and β–effects of the 15-hydroxyl group [15]. A γ-deshielding effect (δ +0.27) for 15α-OH in comparison to δ −0.06 for 15β-OH has been already reported in 15-hydroxyandrostanes [16] suggesting the position of 15-OH in compound **V** as α-orientation. Confirmation of this observation resulted from the measurement of optical rotation [17].

These results proved the ability of *A. strictum* to mediate 15α-hydroxylation. However, no transformation of the decanoyl side chain was detected except an ester hydrolysis. However, 17β-hydroxyl oxidation by *A. strictum* has not been observed before.

15α-Hydroxylation of estrane-based steroids has been reported when using *Aspergillus carneus* [17], *Penicillium* sp. [17], some strains of *Fusarium* sp. [3, 17], and *Glomerella cingulata* [17]. It was also observed that several species of *Fusarium* sp., *Giberella* sp., and *Glomerella* sp. have the ability to mediate the same transformation reaction of various types of steroids [3, 5, 6, 17]. In addition to our previous work [12, 13], we describe *A. strictum* PTCC 5282 as a suitable biocatalyst for transformation of some selected sites in steroids D-ring.

The authors acknowledge Dr. Gholamreza Zarrini regarding fungal studies. They also thank Mr. Afshin Dalvandi for his kind collaboration during spectroscopic work. The helpful advice of Dr. Helmut Brandl (University of Zürich, Zürich, Switzerland) is also acknowledged.

Experimental

Chemicals

Nandrolone decanoate was kindly donated by Iran Hormone pharmaceutical Co. (Tehran, Iran), which had been purchased

from Gedeonrichter (Budapest, Hungary). Sabouraud dextrose agar and broth were purchased from Merck (Darmstadt, Germany). All reagents and solvents were of analytical grade.

Instruments

Melting point (m.p.) was determined on a Reichert-Jung hot stage melting point apparatus (Leica AG, Reichert Division, Vienna, Austria). Optical rotation was measured in 1-dm cells on a Perkin-Elmer 142 automatic spectropolarimeter (Perkin Elmer, Beaconsfield, UK). ^{1}H- and ^{13}C-nuclear magnetic resonance (NMR) spectra were recorded using FT-NMR Varian Unity plus spectrometer at 400 and 100 MHz (Varian Inc., Palo Alto, CA, USA), respectively, in $CDCl_3$ with tetramethylsilane (TMS) as internal standard. Chemical shifts (δ) were given in parts per million (ppm) relative to TMS. Coupling constant (J) were given in Hertz (Hz). Infrared (IR) spectra were recorded on a Magna-IR 550 Nicolet FT-IR spectrometer (Nicolet, Madison, WI, USA). Mass spectra (MS) were obtained with a Finnigan MAT TSQ-70 instrument (Finnigan MAT, San Jose, CA, USA) by electron impact (EI) at 70 eV.

Fungal strain

Acremonium strictum PTCC 5282 [12] was maintained at 4°C on Sabouraud 4% dextrose agar slant and freshly subcultured before use in transformation experiments. The organism was transferred to fresh medium every two months.

Incubation conditions

Ten 500-mL Erlenmeyer flasks, each containing 100 mL of liquid medium of Sabouraud 2% dextrose broth, were inoculated with freshly obtained spores from agar slant cultures and incubated for 12 h at 25°C in a rotary shaker (150 rpm). Spores were collected with sterile normal saline solution containing 0.1% Tween 80. Nandrolone decanoate (1 g) was dissolved in 20 mL of absolute ethanol and 2 mL of the ethanol solution was added to each 500 mL Erlenmeyer flask; incubation continued for 6 days under the same conditions. Sterile controls were processed similarly.

Biotransformation, products isolation, and purification

At the end of the cultivation period, media were extracted with chloroform and the extract was washed with water, dried over sodium sulfate, and evaporated under reduced pressure. Thin-layer chromatography (TLC) (chloroform/acetone, 8:2).was used to analyze the results of bioconversion. Metabolites were visualized under an UV lamp at 254 nm. Preparative TLC on silica gel G (Kieselgel 60 $HF_{254+366}$, Merck) glass plates ($20 \times 20 \times 0.5$ cm) with thickness of 0.5 mm was used to purify the metabolites, again using above mentioned solvent system. All metabolites were crystallized from methanol.

Analytical experimental data

The analytical data of the compounds are mentioned as follows in respective order:

Estr-4-en-3,17-dione ***II***

Yield 20.4%; m.p. 168–170°C, $[\alpha]_D$ + 138° (MeOH), lit [18] m.p. 168–171°C, $[\alpha]_D$ + 139.2°; IR ν_{max} 2929, 1735, 1666, 1615 cm^{-1}; MS (EI) m/z (%) 272 (15) (M^+, $C_{18}H_{24}O_2$), 230 (10), 186 (10), 149 (28), 97 (33), 85 (65), 83 (100); ^{1}H-NMR ($CDCl_3$) δ 0.92 (3H, s, H-18), 5.86 (1H, s, H-4); R_f in chloroform/acetone (8:2): 0.77.

17β-Hydroxyestr-4-en-3-one ***III***

Yield 16.6%; m.p. 112–115°C, $[\alpha]_D$ + 55° (MeOH), lit [19] m. p. 111–112 and 123–124°C (dimorphic crystals), $[\alpha]_D$ + 55.2°; IR ν_{max} 3451, 2934, 1675, 1630 cm^{-1}; MS (EI) m/z (%) 274 (70) (M^+, $C_{18}H_{26}O_2$), 256 (18), 215 (25), 197 (22), 173 (24), 147 (36), 110 (100), 79 (36); ^{1}H-NMR ($CDCl_3$) δ 0.82 (3H, s, H-18), 3.67 (1H, t, J = 8.8 Hz, H-17), 5.83 (1H, s, H-4); R_f in chloroform/acetone (8:2): 0.54.

15α-Hydroxyestr-4-en-3,17-dione ***IV***

Yield 4.9%; m.p. 195–199°C, $[\alpha]_D$ + 155° (MeOH), lit [17] m.p. 200–201°C, $[\alpha]_D$ + 162°; IR ν_{max} 3450, 2920, 1652, 1625 cm^{-1}; MS (EI) m/z (%) 288 (100) (M^+, $C_{18}H_{24}O_3$), 272 (13), 260 (22), 215 (18), 160 (12), 147 (17), 110 (19), 90 (21); ^{1}H-NMR ($CDCl_3$) δ 0.95 (3H, s, H-18), 3.02 (1H, dd, J = 8.2 Hz, J = 8 Hz, H-16), 4.43 (1H, dd, J = 8.2 Hz, J = 7.8 Hz, H-15), 5.83 (1H, s, H-4); R_f in chloroform/acetone (8:2): 0.39.

15α,17β -Dihydroxyestr-4-en-3-one ***V***

Yield 5.6%; m.p. 138–141°C, $[\alpha]_D$ + 91.5°, lit [17] m. p. 136–139°C, $[\alpha]_D$ + 95°; IR ν_{max} 3404, 2928, 1663, 1620 cm^{-1}; MS (EI) m/z (%) 290 (30) (M^+, $C_{18}H_{26}O_3$), 272 (51), 218 (12), 163 (22), 149 (55), 110 (100), 97 (52), 81 (41); ^{1}H-NMR ($CDCl_3$) δ 0.83 (3H, s, H-18), 3.92 (1H, dd, J = 8 Hz, H-17), 4.12 (1H, m, H-15), 5.82 (1H, s, H-4); R_f in chloroform/acetone (8:2): 0.08.

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