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Biotransformation of 6-dehydroprogesterone with *Aspergillus niger* and *Gibberella fujikuroi*

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ABSTRACT

Microbial transformation of 6-dehydroprogesterone (**1**) with *Aspergillus niger* yielded three new metabolites, including 6β-chloro-7α,11α-dihydroxypregna-4-ene-3,20-dione (**2**), 7α-chloro-6β,11α-dihydroxypregna-4-ene-3,20-dione (**3**), and 6α,7α-epoxy-11α-hydroxypregna-4-ene-3,20-dione (**4**), and two known metabolites; 6α,7α-epoxypregna-4-ene-3,20-dione (**5**), and 11α-hydroxypregna-4,6-diene-3,20-dione (**6**). Compounds **2**, and **3** contain chlorohydrin moiety at C-6, and C-7, respectively. The biotransformation of **1** with *Gibberella fujikuroi* yielded a known compound, 11α,17β-dihydroxyandrosta-4,6-dien-3-one (**7**).

1. Introduction

Whole-cell biocatalysis provides efficient route for the synthesis of diverse and value-added chemicals [1]. Biocatalysis have advantages over conventional chemical synthesis due to its selective nature, environmental acceptability, and mild condition [2]. Whole-cell biocatalysis are comparatively economical as compared to pure enzymes [3]. A number of novel reactions, such as Baeyer-Villiger oxidation, structural rearrangements, epoxidation, and Knoevenagel reaction have been reported either through whole-cell biotransformation or by using pure enzyme [4–9]. Among whole-cell biocatalysis, fungi have been used extensively for the regio- and stereo-selective synthesis of chiral compounds [10–16].

6-Dehydroprogesterone (**1**), $C_{21}H_{28}O_2$, is a synthetic derivative of progesterone. Progesterone regulates several functions in body [17]. Plant pathogenic fungus *Botryodiplodia theobromae* was used for the synthesis of 6-dehydroprogesterone from progesterone [18]. *Aspergillus niger*, and *Gibberella fujikuroi* were selected for the microbial transformation of **1**, as these organisms are widely available, easy to maintain and grow, and used extensively for the structural transformation of different classes of compounds [19–21]. 6-Dehydroprogesterone (**1**) was selected for biotransformation, as it belongs to progestational hormones and contains multiple sites for reactions. Aim of this study was to synthesize novel structural analogues of 6-dehydroprogesterone (**1**) by using whole cell cultures of *A. niger*, and *G. fujikuroi*.

This manuscript is in continuation of our studies on the biotransformation of steroidal bioactive compounds into their novel analogues [22–26]. We subjected 6-dehydroprogesterone (**1**) to microbial transformation with *Aspergillus niger*, and *Gibberella fujikuroi*. Substrate **1** yielded compounds **2**–**6** on incubation with *Aspergillus niger*, whereas metabolite **7** was produced on incubation with *Gibberella fujikuroi* (Figs. 1 and 2). Metabolites **2**–**4** were found to be new. Chlorohydrin moiety was characterized in compounds **2** and **3**, which is an interesting finding. This study on biotransformation of 6-dehydroprogesterone has yielded two novel analogues, which not only expanded the available chemical space of progesterone class, but also created possibilities for finding new biological activities. To the best of our knowledge, this is the first report of microbial transformation of 6-dehydroprogesterone (**1**) with *Aspergillus niger*, and *Gibberella fujikuroi*.

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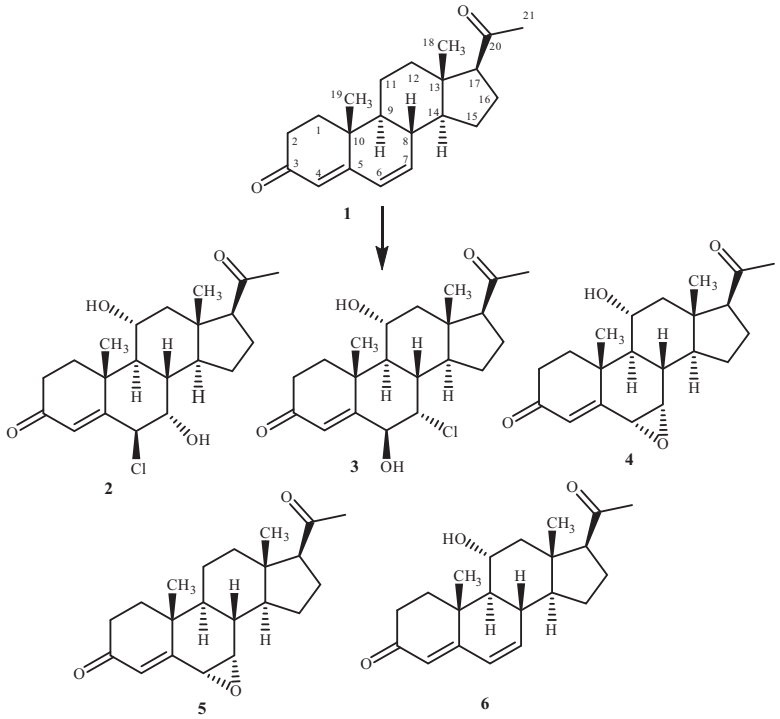

Fig. 1. Compounds **2–6** obtained through whole cell biotransformation of 6-dehydroprogesterone (**1**) by *Aspergillus niger*.

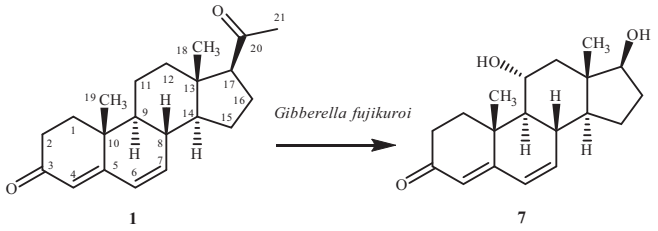

Fig. 2. Compound **7** was obtained through whole cell biotransformation of 6-dehydroprogesterone (**1**) by *Gibberella fujikuroi*.

2. Experimental

2.1. General experimental conditions and instruments

6-Dehydroprogesterone (**1**) was procured from Hangzhou Dayangchem (China). Sabouraud dextrose agar, used for growing fungal cultures, was obtained from Merck KGaA (Cat No. 146392, Germany). Thin layer chromatography (TLC) was carried out on precoated TLC plates (PF_{254}, Merck KGaA, Germany). Flash silica gel was used for column chromatography (200–400 mesh size, E. Merck, Germany). Compounds were purified on recycling preparative HPLC–LC–908 (Japan), equipped with JAIGEL–ODS–L–80 column. Buchi M-560 apparatus was used for melting points measurement. Optical rotations were measured with a JASCO P–2000 polarimeter. UV spectra were recorded in methanol on Shimadzu UV 240 spectrophotometer (Shimadzu Corporation, Tokyo, Japan). ^{1}H and ^{13}C NMR spectra were recorded on Bruker Avance-NMR spectrometer. Jeol JMS-600H (Japan) mass spectrometer (EI-MS) was used to record the mass spectra of isolated compounds. HRESI-MS analysis were performed on QSTAR XL mass spectrometer (Applied Biosystems, USA). IR absorbances were measured on Vector 22 spectrophotometer (Bruker). Single-crystal X-ray diffraction data was collected on Bruker Smart APEX II fitted with CCD 4-K diffractometer [27]. Data reductions were performed by using SAINT program, followed by structure solution (direct method), and refinement by full-matrix least squares on F2 by using the SHELXTL-PC package [28,29]. The figures were plotted with the aid of ORTEP program [30]. All the reagents and solvents used in this experiment were of analytical grade.

2.2. Fungal cultures and medium

The fungal culture *Aspergillus niger* (ATCC 10549) and *Gibberella fujikuroi* (ATCC 10704) were purchased from American Type Culture Collection (ATCC). The fungal strains were grown on Sabouraud dextrose agar and stored at 4 °C.

The growth medium for *Aspergillus niger* was prepared by mixing the following ingredients, glucose (10.0 g), yeast extract (5.0 g), peptone (5.0 g), KH_2PO_4 (5.0 g), NaCl (5.0 g), and glycerol (5.0 mL) in one liter of distilled water. Similarly for *Gibberella fujikuroi*, the culture medium was prepared by mixing of glucose (80.0 g), KH_2PO_4 (5.0 g), NH_4NO_3 (1.0 g), and $MgSO_4{\cdot}7H_2O$ (1.0 g) in one liter of distilled water.

2.3. Incubation of 6-dehydroprogesterone (*1*) with Aspergillus niger

Four liter culture medium was prepared for *Aspergillus niger*, and distributed equally in 40 Erlenmeyer flasks of 250 mL *i.e.*, each flasks contain 100 mL of culturing medium. Seed flaks were prepared by inoculating with *A. niger* spores and incubated at 26 °C for 3 days. Within two to three days, fungus growth was observed. The remaining flasks were inoculated by transferring spores from seed flasks. 6-Dehydroprogesterone (**1**) (300 mg) was dissolved in methanol (20 mL), and distributed to 40 flasks containing 3–4 days old cultures of *A. niger* (7.5 mg in each flask). These flasks were placed over rotary shaker for fermentation for 12 days at 26 °C. On completion of fermentation reaction, the fungal mass was filtered off, and extracted three times with CH_2Cl_2 (24 L). The extract was dried over Na_2SO_4 (anhydrous) and concentrated under reduced pressure to obtain a brown gum (2.1 g). This gum was