Biotransformation of anabolic drug Dianabol with *Rhizopus oryzae*

**Abstract.** Microbial biotransformation technique is an excellent approach for the synthesis of stereo-, enantio-, chemo-, and regio-selective/specific analogues of existing steroidal and non-steroidal drugs by using bacteria, fungi, algae, actinomycetes, yeast, and plants and animals cell cultures. This technique is effectively used to synthesize compounds whose structures resemble to the substrates (parent drugs) without using protecting/deprotecting steps. In the current study, an anabolic-androgenic steroid (AAS) based drug, methandienone (dianabol) (1) was incubated with the filamentous fungi *Rhizopus oryzae* ATCC 11145 for twelve days under ambient reaction conditions (at room temperature and neutral pH) by using aqueous media. Therefore, the present study has successfully helped to produce the structural analogues of inert steroidal anabolic drug dianabol (1) without using expensive and toxic chemicals. This yielded five known structural analogues of dianabol (1), i.e., 17β,11β-dihydroxy,17α-methyl-androsta-1,4-diene-3-one (2), 17β-hydroxy,17α-methyl-androsta-1,4-diene-3,11-dione (3), 17β,6β-dihydroxy,17α-methyl-androsta-1,4-diene-3-one (4), 17β,6β-dihydroxy,17α-methyl-androsta-4-ene-3-one (5), and 17β-hydroxy,17α-methyl-androsta-4,6-diene-3-one (6). Structures of transformed products 2-6 were determined through 1H-NMR, and FAB-MS spectroscopic techniques.

**Key words:** microbial biotransformation, fungi, *Rhizopus oryzae*, anabolic-androgenic drug, Dianabol.

**Introduction**

Derivatization of organic compounds through conventional synthetic methodologies is a difficult task, as they typically involve the use of expensive and toxic chemicals, and extreme pressure, temperature and pH during the transformations, along with the protection/deprotection steps. In contrast, microbial biotransformation is a robust approach that can effectively modify the structures of almost all classes of organic compounds. This technique is generally performed at ambient temperature and pressure, and neutral pH by using aqueous media. Microbial biotransformation technique is therefore, an efficient way to synthesize regio-, chemo-, and stereo-specific/selective derivatives of existing drugs. The chemical conversion of substrates is catalyzed by various enzymes (biological catalysts) without using protection, deprotection, and functional group activation steps. At present microbial biotransformation is recognized as a green chemistry approach, because it generates less toxic wastes as compared to chemical syntheses. Currently the use of microbial biotransformation is increasing significantly in the production of pharmaceutical products, i.e., vitamins, hormones, enzymes, enzyme inhibitors, vaccines, *etc.* as it preserves the original skeleton of starting material during the formation of products, making it important for industries [1-7].

Methandrostenolone/methandienone (1) is an anabolic-androgenic steroidal drug, used to improve physical performances, and increase muscles without gaining fat. Previously, it was sold under the brand name of dianabol (1) in USA and Germany. Like other steroids, methandienone also has some side effects, including acne, increased hair growth on body, change in voice, estrogenic effects, and liver damage [8, 9]. It is therefore, important to synthesize its analogues by environmentally friendly methods.

In continuance of our biocatalytic studies of steroidal-based compounds/drugs [5-7, 10-13], we
report here biotransformation of dianabol (1) with Rhizopus oryzae under mild reaction conditions. This yielded five known transformed products (Figure-1), \(17\beta, 11\beta\)-dihydroxy, \(17\alpha\)-methyl-androsta-1,4-diene-3-one (2), \(17\beta\)-hydroxy,\(17\alpha\)-methyl-androsta-1,4-diene-3,11-dione (3), \(17\beta,6\beta\)-dihydroxy,\(17\alpha\)-methyl-androsta-1,4-diene-3-one (4), \(17\beta,6\beta\)-dihydroxy, \(17\alpha\)-methyl-androsta-4-ene-3-one (5), and \(17\beta\)-hydroxy,\(17\alpha\)-methyl-androsta-4,6-diene-3-one (6). Structures of metabolites 2-6 were identified through \(^1\)H-NMR, and FAB-MS spectroscopic techniques.

Materials and methods

Fungi. Fungal cell culture e.g., Rhizopus oryzae ATCC 11145 was purchased from ATCC (American Type Culture Collection, USA). Fungal cell culture was grown on SDA at 3-4 °C.

Media. Five liters of media for the growth of \(R.\) oryzae was prepared by using following ingredients: Glucose (50 g), NaCl (25 g), peptone (25 g), KH\(_2\)PO\(_4\) (25 g), and glycerol (50 mL) in five liters of distilled water.

Ingredients for the preparation of media were acquired from Scharlau Chemicals and Reagents (Spain), VWR Chemicals (USA), Dae-Jung Chemicals and Metals Company Limited (Korea), Sigma-Aldrich (Germany), and Oxoid Limited (England).

General. Dianabol (1), \((m/z = 300.4, C_{20}H_{28}O_2)\), was procured from the Shenzhen Simeiquan Biotechnology Company Limited, (China). Silica coated (PF\(_{254}\)) TLC plates (Merck KGaA, Germany) were used to determine the transformations, and purity of compounds. Fractions were obtained by performing silica gel (70–230 mesh) (E. Merck, Germany) column chromatography. Final purifications of fractions were performed via recycling reverse phase HPLC (LC–908, YMC L-80) using methanol/water. The \(^1\)H-NMR spectra of compounds 1-6 were run in CDCl\(_3\), on the Bruker Avance-NMR (Bruker, Switzerland). FAB-MS of compounds 1-6 were performed on the Joel JMS H×110 mass spectrometer (Joel, Japan).

Fermentation of Dianabol (1). Media (5 L) was prepared by aforementioned recipe. Media (400 mL) was transferred to each 20 flasks of 1 L. Each flask was cotton plugged, and autoclaved. All the flasks were then cooled at room temperature. Media was inoculated with the culture of \(R.\) oryzae under sterilized conditions, and placed for four days on rotary shaker at 25 ºC. After maximum growth of \(R.\) oryzae, 2 g of dianabol (1) was dissolved in 20 mL of methanol, and fed 1 mL in each \(R.\) oryzae-containing flask. These flasks were again placed on rotary shaker at 25 °C for twelve days.

Extraction of Transformed Products. After incubation, EtOAc (ethyl acetate) was added in each flask. Flasks were filtered, and rinsed with EtOAc. The filtrate was extracted three times with EtOAc, and biomasses were discarded. Sodium sulfate was added in each extracted flask to absorb water, and filtered. The extracted was then evaporated by using rotary evaporator. As a result, a brown color gummy crude extract was obtained.

Purification of Transformed Products. Crude extract was fractionated by silica-gel column chromatography using hexanes-acetone mixture (5-100%), passing 400 mL at each mixture concentration. TLCs were taken for each fractions. TLCs were stained by ceric sulfate spraying reagent. Fractions were purified through recycling RP-HPLC using methanol (70%) and water (30%) solvent system.

Results and discussion

\(R.\) oryzae-mediated structural modification of dianabol (1) afforded five derivatives, presented on Figure 1.

Their structures were determined by comparing their \(^1\)H-NMR and FAB-MS data with the spectral data reported in the literature.

Metabolite 2 showed its [M-H]\(^+\) at \(m/z\) 315.3 in the FAB-MS (+ve mode). Newly appeared H-11 of compound 2 was observed at \(\delta\) 4.40, \(d (J_{\alpha,11} = 2.22\) Hz). Compound 2 was previously reported by our research group [14].

Transformed product 3 showed its [M+H]\(^+\) at \(m/z\) 315.1 in the FAB-MS (+ve). \(^1\)H-NMR spectrum of compound 3 was distinctly similar to compound 2. Signals for methylene protons (H-12) were found missing in the \(^1\)H-NMR spectrum of 3. Compound 3 was also previously reported by our research group [14].

[M-H]\(^+\) of compound 4 was observed at \(m/z\) 315.2 in the FAB-MS (-ve mode). Newly appeared H-6 of compound 4 was observed at \(\delta\) 4.53, \(d (J_{\alpha,6} = 2.18\) Hz) in the \(^1\)H-NMR spectrum. Compound 4 was previously reported by our research group [14].

Derivative 5 displayed its [M-H]\(^+\) at \(m/z\) 317.2 in the FAB-MS (-ve mode). Newly appeared H-6 of compound 5 was observed at \(\delta\) 4.53, \(d (J_{\alpha,6} = 2.22\) Hz) in the \(^1\)H-NMR spectrum. Signals for olefinic protons H-1 and H-2 were found missing in the \(^1\)H-NMR spectrum of 5 [15].
Compound 6 showed its [M+H]\(^+\) at 301.0 in the FAB-MS (+ve). Olefinic protons H-1 and H-2 were found missing while new downfield signals for olefinic protons H-6, and H-7 were observed at δ 6.09 s in the \(^{1}H\)-NMR spectrum of 6 [16].

**Conclusion**

In the present study biotransformation of dianabol (1) was performed at ambient reaction conditions with *R. oryzae*. This afforded five known structural analogues of anabolic drug 1. The study indicates that *R. oryzae* was able to catalyze β-hydroxylation at C-6, and C-11 of drug 1. Dehydrogenation, reduction, and ketone formation was also observed during the transformation of dianabol (1). In future, transformed products 2-6 will be evaluated against various biological activities. These derivatives will also be studied at enzymatic levels for their mechanism of production.

**References**


**Information about authors:**

Mahwish Siddiqui – PhD at H.E.J. Research Institute of Chemistry (Karachi, Pakistan, e-mail: mahwish.siddiqui88@gmail.com)

Atia-Tul-Wahab – Professor at ICCBS, University of Karachi (Karachi, Pakistan, e-mail: tulwahab@yahoo.com)

Kudaibergenova Bates – PhD, Acc.professor at Al-Farabi Kazakh National University (Almaty, Kazakhstan, e-mail: bateskudaibergenova@yahoo.com)

Zharylkasyn Abilov – Professor at Al-Farabi Kazakh National University (Almaty, Kazakhstan, e-mail: abilov229@mail.ru)

Choudhary, Mohammed Iqbal – Director and Professor of Bioorganic and Natural Product Chemistry at the International Center for Chemical and Biological Sciences (H. E. J. Research Institute of Chemistry and Dr. Panjwani Center for Molecular Medicine and Drug Research), University of Karachi (Karachi, Pakistan, e-mail: iqbal.choudhary@iccs.edu)