



A Whole-cell Biocatalysis Application of Steroidal Drugs

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DOI: <http://dx.doi.org/10.13005/ojc/290201>

(Received: April 10, 2013; Accepted: May 27, 2013)

ABSTRACT

Biocatalysis is also widely applicable for enhancement of bioprocess productivity, selectivity of target reactions and production of valuable pharmaceutical ingredients, precursors and key intermediates. Microorganisms and cell suspension cultures of plant have found wide application in biotransformations of steroidal drugs in order to obtain high regio- and stereoselective products. Studies of steroid modifications catalyzed by microbial or plant cell cultures represent a well-established research approach and methodology in white biotechnology. Bioconversion can be done at the position of steroid molecule hardly available for chemical agents; functionalization of the molecule can be performed stereospecifically; several reactions can be completed in one biotechnological step.

Key words: Steroidal drugs, Microbial transformation, Hydroxylation, Whole-cell biocatalysis, Plant cell cultures.

INTRODUCTION

Biocatalysis is an increasingly important tool in organic synthesis. Biotransformations with whole cells are extensively employed as an industrial method to synthesize many steroidal drugs and chiral building blocks, and to modify natural products with potent biological activities. Such studies are primarily useful in the generation of hydroxylated metabolites for drug toxicity studies. Microorganisms such as fungi, bacteria and yeast possess multi-enzyme systems which have significant regio- and stereo-selectivity. Thus,

microbial transformation technique is increasingly being used as one of the feasible approaches by far for structurally modifying natural and synthetic compounds. Microbial hydroxylation of steroid is industrially important since it involves the synthesis of many intermediates necessary for the synthesis of many steroidal drugs¹⁻².

Microbial transformation has also found wide applications in biotransformation of steroids in order to obtain high regio- and stereoselective products. They have been utilized successfully as in vitro models to mimic and predicts the metabolic

fate of drugs and other xenobiotics in mammalian systems. They can transform fine chemicals extensively through environmental friendly and cost effective way. The transformation is mostly carried out by fungi including hydroxylation, oxidation and reduction of alcohols, ketones and C=C bond²⁻³.

Steroids (Greek, stereos = solids) are solid alcohols that are widely distributed in the animal and plant kingdom. The basic skeleton of steroids consists of 17 carbon atoms arranged in the form of a perhydrocyclopentanophenanthrene (Fig. 1). They include great variations in structure and encompass compounds of vital importance to life, such as cholesterol, the bile acids, sex hormones, vitamin D, corticoid hormones, cardiac aglycones, antibiotics, and insect molting hormones. Some of the most potent toxins are steroidal alkaloids. Steroidal compounds are responsible for various important biological functions in the cells, e.g. steroids of androstane, pregnane and estrane series exhibit various hormonal activities; bile acids are essential for lipid digestion and absorption and cardiac aglycones are used for heart failure treatment. Sterols are important constituents of cell membranes, essential for their stability, cell growth, and proliferation and are precursors of bile acids and hormonal steroids. Some steroidal compounds

are used as an important source of raw materials for the production of steroids of the androstane, pregnane and estrane series. Totally, about 300 approved steroid drugs are known to date, and this number tends to grow. Steroid pharmaceuticals are ranked among the most marketed medical products and represent the second large category next to antibiotics. The annual production of steroid drugs exceeded 1,000,000 tons and the global market is around US\$ 10 billion⁴⁻⁵.

There is a well-known example that chemical steroid synthesis involves 31 reaction steps to yield 1 g cortisone from 615 g of deoxycholic acid (purified from beef bile). Transformation of simple natural precursor molecule using *Rhizopus arrhizus* ATCC 11145 and *Aspergillus niger* ATCC 9142 brought down cortisone manufacturing costs from \$ 200 (1949) to \$ 1 (1979) and reduced 31 chemical steps to 11 steps. Basically, microbial transformations require mild conditions which are of importance when working with molecules unstable at high temperatures or extremal pH values. There is a possibility of easier isomer separation in bioconversion. And, in general, biotechnologies are more ecologically friendly as compared with chemical synthesis⁶⁻⁷.

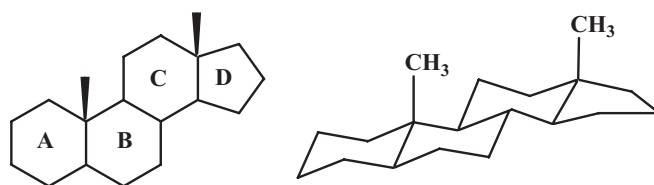


Fig. 1: Basic steroidal skeleton

Microbial catalysis was established to be the most efficient way to solve another troublesome task for steroid chemistry, which is oxidation of inactivated carbon. Microorganisms such as fungi and bacteria are capable of introducing one or more hydroxyl groups at different positions of the gonane core or side chain are known. Hydroxylation is one of the most important reactions of steroid functionalization. Hydroxylated steroids often express a higher biological activity as compared with their less polar non-hydroxylated analogs. For example, 7 α -hydroxy derivatives of DHEA exhibited

severalfold higher immunoprotective and immunoregulatory properties as compared with non-hydroxylated DHEA; a few more hydroxylated derivatives of ethisterone displayed tyrosinase inhibitory effects; some novel hydroxy derivatives of HMPD (20-hydroxymethylpregna-1,4-diene-3-one) expressed cytotoxicity towards HeLa cancer lines unlike non-hydroxylated substrate⁶⁻⁸.

The research efforts in microbial technology were triggered around 1950, with the announcement of the pharmacological effects of

cortisol and progesterone and with the identification of 11 α -hydroxylation activity of a *Rhizopus* species, a decisive step in the development of the practical synthesis of steroids with useful biological activity. The great versatility of microbial systems in the production of commercially valuable steroids for the pharmaceutical industry is now well recognized. The areas which are now receiving attention in microbial technology are: application of newer concepts of genetic engineering of microbes with improved characteristics, solubility improvement for carrying out biotransformation of substrates that are insoluble in water, immobilization of enzymes or whole cells in a suitable matrix for economic utilization, development of a continuous process for better and economic product recovery; and manipulation of culture media for improvement in product yields by use of cyclodextrin. Several biotransformation of steroidal drugs through microorganisms and plant cell cultures have been reported³.

During recent years our research has been focused on the structural modifications of bioactive steroids by using various microorganisms and plant cell cultures, in order to obtain biologically potent compounds with diverse structures and various US patents have also been granted on the methods of producing bioactive metabolites through fermentation²²⁻³⁰.

Biotransformation of phytosterols

Phytosterols, together with sapogenins such as diosgenin, hecogenin and solasodine, are established in the pharmaceutical industry as starting materials for the production of steroidal hormones. It is a well-known fact that a wide range of microorganisms is capable of utilizing sterols such as cholesterol and phytosterols as the sole carbon source for the growth and propagation. It is also well documented that in the sterol degrading microorganisms, the ring nucleus and the side chain are metabolized by different enzyme systems simultaneously and independently⁹.

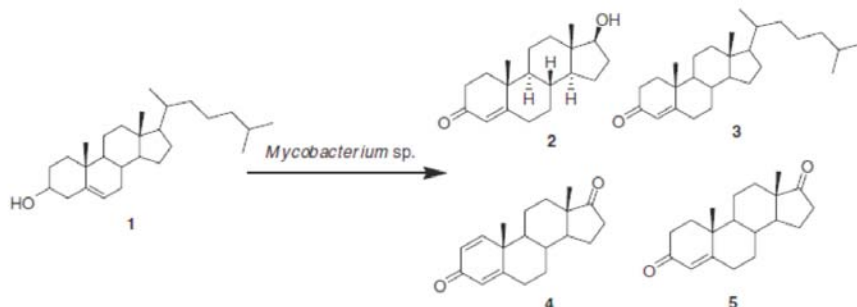


Fig. 2: Microbial transformation of cholesterol (1)

Cholesterol (1) is known as animal sterol; sitosterol, stigmasterol, campesterol, and brassicosterol are abundant plant sterols; the so-called phytosterols are mixtures of plant sterols mainly of soya origin, or produced from tall oil or pitch; ergosterol is a major sterol of yeasts and fungi. Since the 1980s, microbial transformation of phytosterol remains a focus of research in the field of steroids. The production of testosterone (2), a male sex hormone or androgen, from cholesterol (1) via a single step microbial transformation process by *Mycobacterium* sp. NRRL B-3805. While incubation of cholesterol (1) with free cells of *Arthrobacter simplex* afforded cholest-4-en-3-one (3), androst-1,4-dien-3,17-dione (ADD) (4) and

androst-4-en-3,17-dione (5) in an aqueous organic solvent two-phase system (Fig. 2)⁹⁻¹¹.

Biotransformation of steroidal drugs

Sex hormones are hormones that affects the reproductive system. They include estrogens, progesterone and androgens (such as testosterone). Androgen is the generic term for any natural or synthetic compound, usually a steroidal hormone, that stimulates or controls the development and maintenance of masculine characteristics in vertebrates. Steroid hormones are also known to control distinct aspects of cell proliferation and tissue differentiation and to regulate signal transduction pathways by binding

to respective intracellular receptors that act as transcription factors to modulate specific gene expression

C-3 glycoside derivatives of 14-hydroxy-5 β , 14 β -pregnane show strong interaction with cardiac glycoside receptor of heart muscle. Such compounds have been mainly obtained by multistep chemical transformations of digitoxine, digitoxigenin and related compounds that contain the 5 β , 14 β -configuration (Fig. 3). Conversely, the 14 α -hydroxylation of progesterone (6) and other steroids by several microorganisms is a well documented fact and several fungi have been shown to introduce, in reasonable yields, a hydroxyl group at the 14 α -position of progesterone and some other steroids; the 14 α -hydroxy configuration can then be transformed chemically to the 14 β -hydroxy substituent normally found in cardioactive steroids. Microbial transformation of progesterone (6) by *Thamnostylum piriforme* yielded 14 α -hydroxyprogesterone (7) and 9 α -hydroxyprogesterone (8). A transformation with *Mucor griseocyanus* afforded 7, 6 β ,14 α -dihydroxyprogesterone (9) and 7 α ,14 α -dihydroxyprogesterone (10). The filamentous fungus, *Aspergillus fumigatus* efficiently hydroxylated 6 producing 11 α -hydroxyprogesterone (11), 15 β -hydroxyprogesterone (12), 7 β -hydroxyprogesterone (13), 7 β ,15 β -dihydroxyprogesterone (14) and 11 α ,15 β -dihydroxyprogesterone (15). Incubation of

progesterone (5) with *Saprolegnia hypogyna* yielded 4-androstene-3, 17-dione (16), testosterone (2) and testolactone (17). Biotransformation of 5 with *Fusarium culmorum* culture yielded a mixture of 15 α -hydroxyprogesterone (18) and 12 β ,15 α -dihydroxyprogesterone (19). However when 5 was fermented with thermophilic bacterium, *Bacillus stearothermophilus*, three monohydroxylated metabolites and a new metabolite, were isolated and identified. These were identified as 20 α -hydroxyprogesterone (20), 6 β -hydroxyprogesterone (21), 6 α -hydroxyprogesterone (22) and 9, 10-sco-4-pregnene-3, 9, 20-trione (23) as shown in Figure 4^{2-3, 12-14}.

Pregnenolone (24) is a major hormone in human nerve tissues and its therapeutic role in repairing defective neurons is well documented. Moreover, it is a precursor of many steroidal hormones. Biological transformation of pregnenolone (24) by *Mucor piriformis* resulted in the isolation of two metabolites, 3 β ,7 α -dihydroxypregn-5-en-20-one (25) and 3 β ,7 α ,11 α -trihydroxypregn-5-en-20-one (26). Fermentation of 24 with the fungus *Botrytis cinerea* yielded two new oxidized metabolites of pregnenolone. These were identified as 3 β ,11 α ,16 β -trihydroxypregn-5-en-20-one (27) and 11 α ,16 β -dihydroxypregn-4-en-3, 20-dione (28). Metabolism of pregnenolone (24) by *Bacillus* strains afforded 25,3 β ,7 β -dihydroxypregn-5-en-20-one (29) and 7-oxopregnenolone (30) as the major metabolites (Fig. 5)²⁻³.

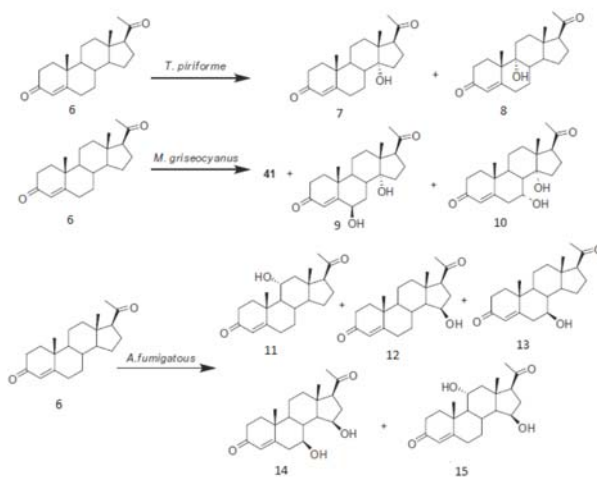


Fig. 3: Microbial transformation of progesterone (6) by *T. piriforme*, *M. griseocyanus* and *A. fumigatus*

Dehydroepiandrosterone (DHEA) (31) is the most dominant hormone in the body. It is the source of all sex and steroid hormones and known as the body's mother hormone. Biotransformation of dehydroepiandrosterone (DHEA) (31) was carried out by a plant pathogen *Rhizopus stolonifer*, which resulted in the production of seven metabolites. These metabolites were identified as

3 β ,17 β -dihydroxyandrost-5-ene (32), 3 β ,17 β -dihydroxyandrost-4-ene (33), 17 β -hydroxyandrost-4-ene-3-one (34), 3 β ,11 β -dihydroxyandrost-4-ene-17-one (35), 3 β ,7 α -dihydroandrost-5-ene-17-one (36), 3 β ,7 α ,17 β -trihydroxyandrost-5-ene (37) and 11 β -hydroxyandrost-4,6-diene-3,17-dione (38) (Fig. 6)¹⁵.

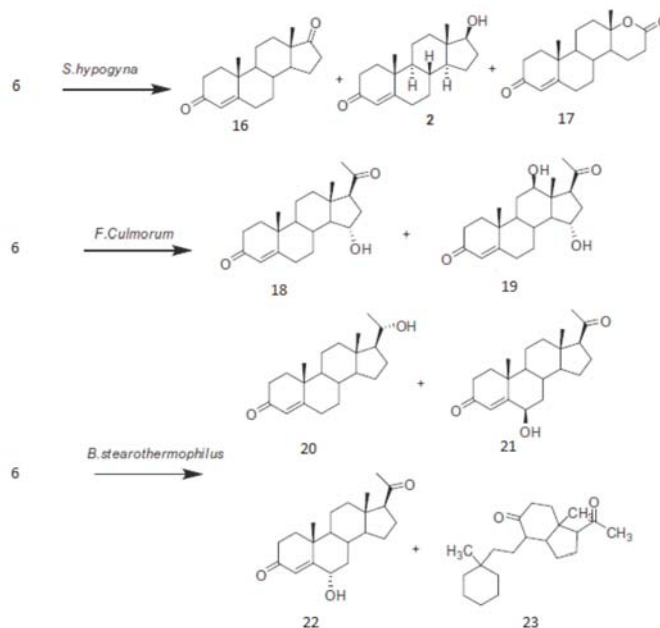


Fig. 4: Microbial transformation of progesterone (6) by *S. hypogyna*, *F. culmorum* and *B. stearothersophilus*

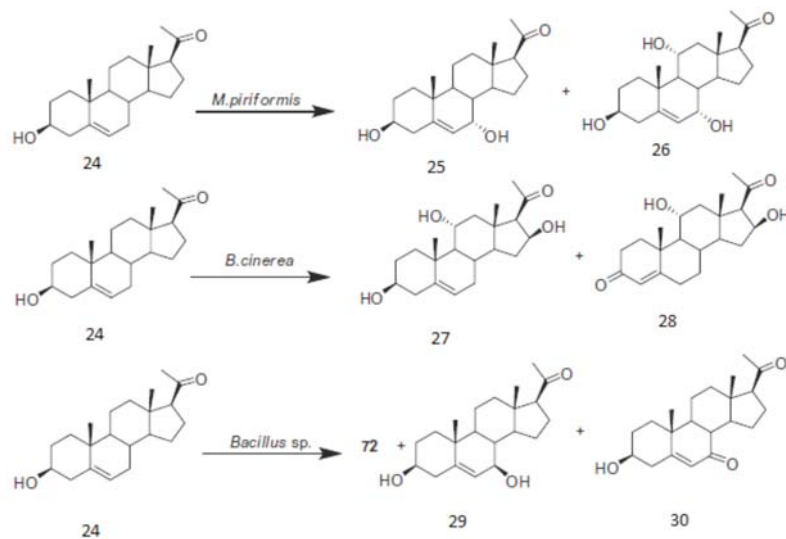


Fig. 5: Microbial transformations of pregnenolone (24) *M. piriformis*, *B. cinerea* and *Bacillus sp.*

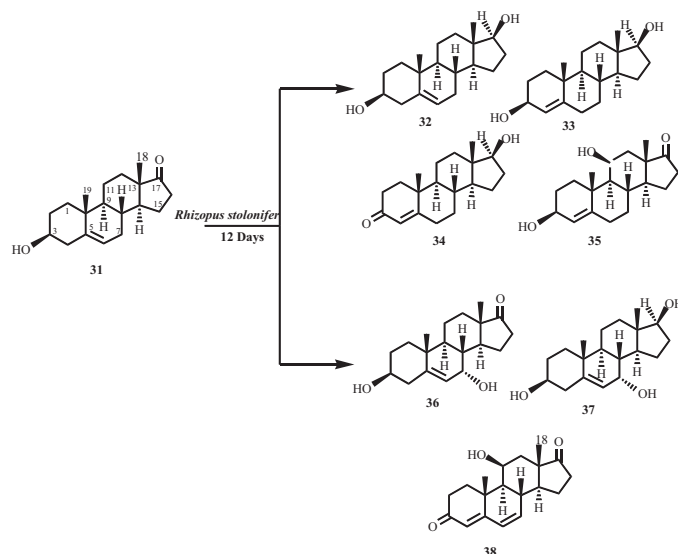


Fig. 6: Fermentation of dehydroepiandrosterone (31) with *Rhizopus stolonifer*

Mesterolone ((1 α , 5 α , 17 β)-17-hydroxy-1-methylandrostan-3-one) (39) is a steroidal drug used in the treatment of conditions caused by deficient endogenous androgen formation, which can develop gradually with increasing age. Microbial transformations of mesterolone (39) has been investigated using *Cephalosporium aphidicola*, *Fusarium lini* and *Rhizopus stolonifer*. Metabolites (1 α ,5 α)-1-methylandrostan-3, 17-dione (40), (1 α ,3 β ,5 α ,17 β)-1-methylandrostan-3, 17-diol (41) and (1 α ,5 α ,15 α)-15-hydroxy-1-methylandrostan-3,17-dione (43) were produced

by *Cephalosporium aphidicola*, while metabolites, (5 α)-1-methylandrostan-1-en-3,17-dione (42), 43, (1 α ,5 α ,6 α ,17 β)-6,17-dihydroxy-1-methylandrostan-3-one (44), (1 α ,5 α ,15 α ,17 β)-15,17-dihydroxy-1-methylandrostan-3-one (45) and (5 α ,15 α ,17 β)-15,17-dihydroxy-1-methylandrostan-1-en-3-one (46) were produced by *Fusarium lini*. Similarly, *Rhizopus stolonifer* yielded metabolites 40, 44, (1 α , 5 α , 7 α , 17 β)-7,17-dihydroxy-1-methylandrostan-3-one (47), (1 α , 5 α ,11 α ,17 β)-11,17-dihydroxy-1-methylandrostan-3-one (48) and 46. These transformations are shown in Figure 7¹⁶.

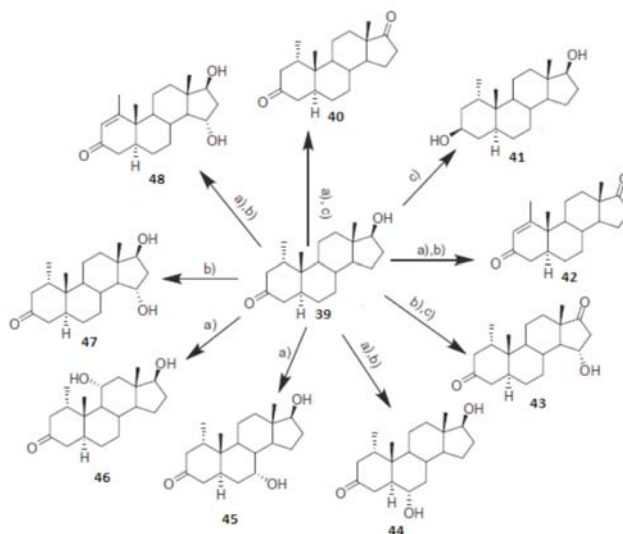


Fig. 7: Microbial transformations of mesterolone (39) by (a) *R. stolonifer*, (b) *F. lini*, and (c) *C.aphidicola*

Danazol (17 β -hydroxy-17 α -pregna-2,4-dien-20-yno-[2,3-d] isoxazole (49) is an orally effective, pituitary gonadotropin inhibitor which is effectively used in the treatment of endometriosis, benign fibrocystic mastitis and precocious puberty. Biotransformation of danazol (49) has been carried out using four different fungi and bacteria to study the transformation of danazol (49). Fermentation of

danazol (17 β -hydroxy-17 α -pregna-2,4-dien-20-yno-[2,3-d] isoxazole (49) with *Fusarium lini*, *Aspergillus niger* and *Cephalosporium aphidicola* afforded 17 β -hydroxy-2-(hydroxymethyl)-17 α -pregn-4-en-20-yn-3-one (50) and 17 β -hydroxy-2-(hydroxymethyl)-17 α -pregn-1,4-dien-20-yn-3-one (51), while the fermentation of 49 with *Bacillus cerus* yielded the metabolite 50 only as shown in Fig. 8².

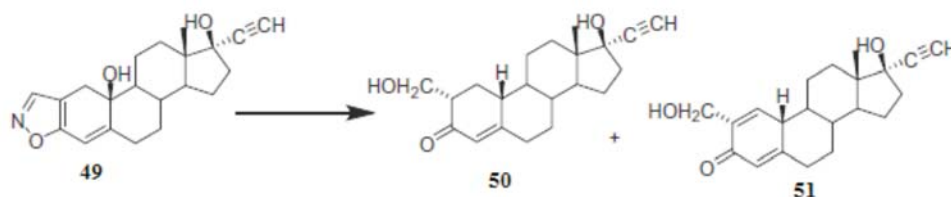


Fig. 8: Biotransformation of Danazol (49)

Prednisone (17 α ,21-dihydroxypregna-1,4-diene-3,11,20-trione) (52) is commonly used in the treatment of asthma, rheumatic disorders, renal disorders and diseases of inflammatory bowel, skin. Gastrointestinal tract. Fungal transformation of prednisone (52) by *Cunninghamella elegans* afforded two metabolites, 17 α ,21-dihydroxy-5 α -pregn-1-ene-3,11, 20-trione (53) and 17 α ,20S,21-trihydroxy-5 α -pregn-1-ene-3, 11-dione (54), while fermentation of 52 by *Fusarium lini*, *Rhizopus stolonifer* and *Curvularia lunata* yielded a metabolite 1,4-pregnadiene-17 α , 20S, 21-triol-3,11-dione (55) (Fig. 9)².

Cortisol (56) is an important anabolic hormone clinically used in topical anti-inflammatory and antiallergic medicines. It is also used in the synthesis of many steroidal hormones. Microbial transformation of cortisol (56) with *Gibberella fujikuroi* yielded 11 β -hydroxyandrost-4-en-3,17-dione (57), while incubation with *Bacillus subtilis* and *Rhizopus stolonifer* afforded 11 β ,17 α ,20,21-tetrahydroxy- (20S)-pregn-4-en-3-one (58). The metabolites 11 β ,17 α ,21-trihydroxy-5 α -pregnan-3, 20-dione (59) and 3 β ,11 β ,17 α , 21-tetrahydroxy-5 α -pregnan-20-one (60) were obtained by fermentation of 56 with *Bacillus cerus* as shown in Figure 10².

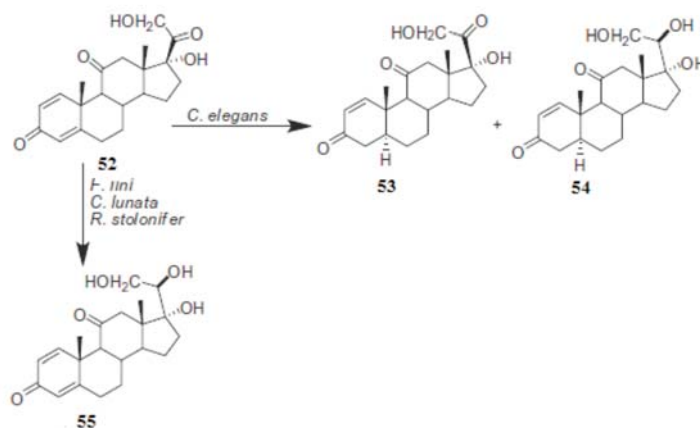


Fig. 9: Microbial transformations of prednisone (52)

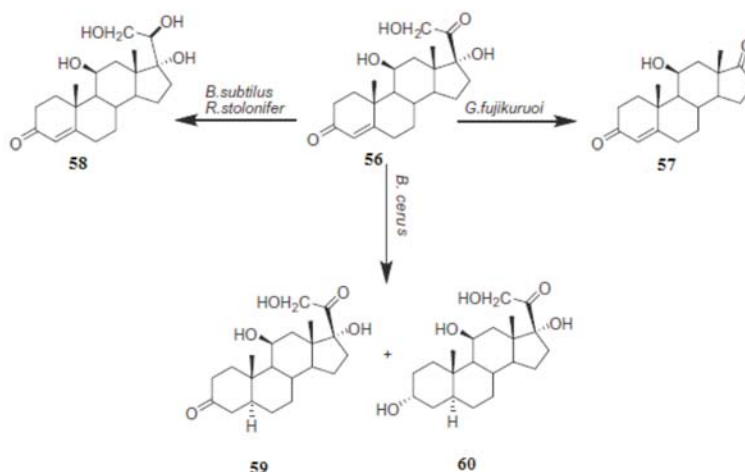


Fig. 10: Microbial transformations of cortisol (56)

Testosterone (17 β -hydroxyandrost-4-en-3-one) (2) is a male sex hormone responsible for the development of secondary male characteristics. The hydroxylation of steroids is important because of its physiological role in mammalian organisms and also for practical reasons. Testosterone (2) was actively transformed by *M. griseocyanus* and *T. piriforme*. *M. griseocyanus* produced a major metabolite, 14 α ,17 β -dihydroxyandrost-4-en-3-one (61) and 14 α -hydroxyandrost-4-en-3, 17-dione (62). Conversely, *T. piriforme* produced lower amounts of 14 α hydroxylated derivatives and yielded 6 β ,17 β -dihydroxyandrost-4-en-3-one (63), 9 α ,17 β -dihydroxyandrost-4-en-3-one (64) and 6 β hydroxyandrost-4-en-3, 17-dione (65). Microbial transformation of testosterone (2) by *Nectria haematococca* afforded 1-ene-testosterone (66) and 11 α -hydroxy-1-ene-testosterone (67) (Fig. 11). Fermentation of 2 with *Fusarium culmorum* yielded 63 and 15 α -hydroxytestosterone (68) as the major metabolites. The fungus *Cephalosporium aphidicola* hydroxylated testosterone (2) to metabolites 61 and 63. The metabolites 63, 68, androst-4-ene-3, 17-dione (69) and 15 α -hydroxyandrost-4-ene-3,17-dione (70) were obtained when compound 2 was metabolized with the fungus *F. oxysporum* var. cubense, while the fungi *Curvularia lunata*, *Pleurotus oestreatus* yielded only the metabolites 69 and 70. The plant pathogenic fungus *Botrytis cinerea* yielded only the metabolite

7 β , 17 β -dihydroxyandrost-3-one (71). Biotransformation of testosterone (2) by *Bacillus stearothermophilus* resulted in the formation of 63, 68, 6 α ,17 β -dihydroxyandrost-4-en-3-one (72), 6 β -hydroxyandrost-4-en-3, 17-dione (73) and 6 α -hydroxyandrost-4-en-3, 17-dione (74). These fermented products are shown in Figure 12².

Tibolone (75), a synthetic steroid that is used for hormone replacement in menopausal women, is metabolized to three primary active agents: 3 α -hydroxytibolone, 3 β -hydroxytibolone and Δ^4 -tibolone (76). It can exhibit estrogenic, progestogenic or androgenic activity, depending primarily on the target tissue involved; for this reason, tibolone is said to have tissue-specific activity and structurally related to 19-nortestosterone derivatives. Tibolone (75) and its metabolites also showed tissue specific inhibition of sulfatase enzyme which could be protective against development of mammary carcinomas, whereas it retains favorable estrogenic effects on bone. Whole cells based biotransformation of tissue-specific hormone tibolone (75) was carried out with *Trichothecium roseum* (ATCC 13411) yielded Δ^4 -Tibolone (76), $\Delta^{1,4}$ -tibolone (77), 15 α -hydroxy- Δ^4 tibolone (78), 12 β -hydroxy- Δ^4 -tibolone (79) and 3 α ,6 α -dihydroxy- Δ^4 -tibolone (80). These metabolized products are shown in Figure 13^{14,17}.

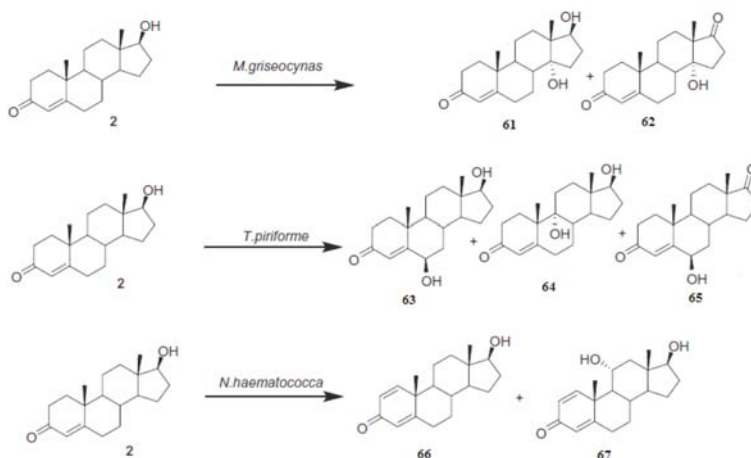


Fig. 11: Biotransformations of testosterone (2) by *M. griseocynas*, *T. piriforme* and *N. haematococca*

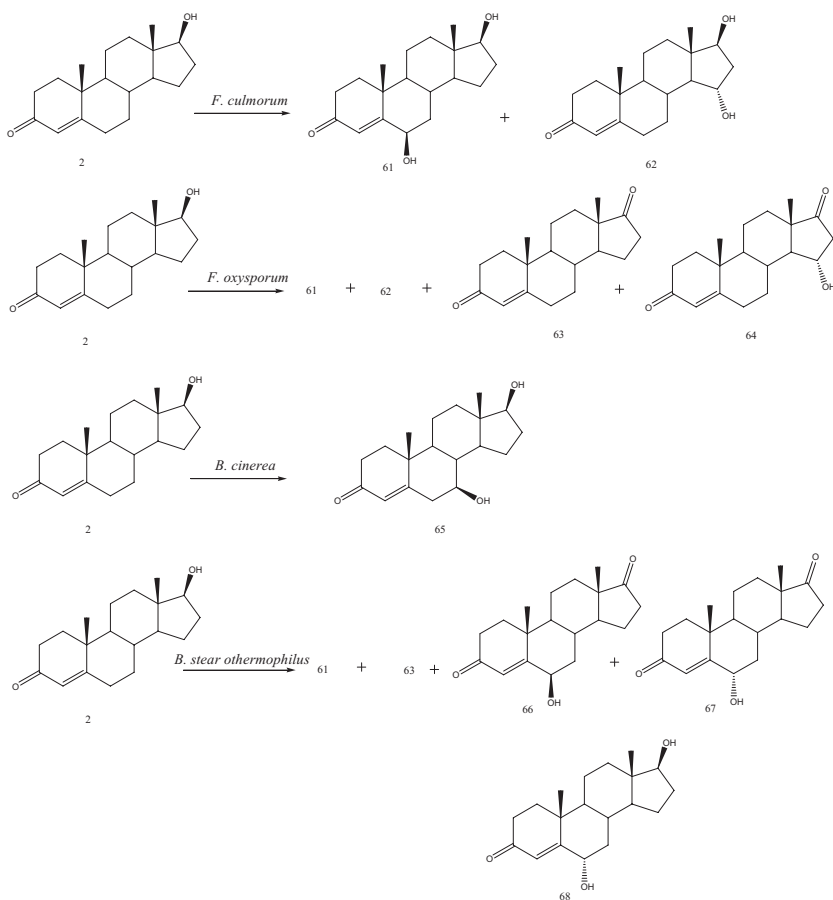


Fig. 12: Biotransformations of testosterone (2) by *F. culmorum*, *F. oxysporum*, *B. cinerea* and *B. stearithrophilus*

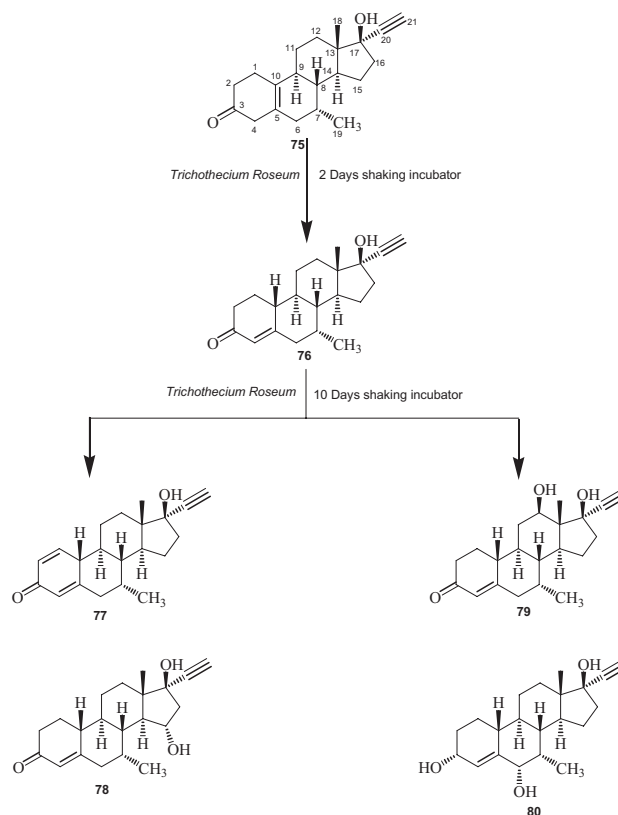


Fig. 12: Biotransformations of tibolone (75) by fungal culture of *Trichothecium roseum*

Ethinodiol diacetate (81) is a semi synthetic steroidal drug, used as an oral contraceptive. It inhibits the ovulation process, and serves as a potent progestin. It provides adequate control of menstrual cyclicity in combination with an estrogen, and thus has a complete contraceptive property, even in low doses. Biotransformation of an oral contraceptive, ethynodiol diacetate (81), by using microbial and plant cell cultures provides an efficient route to the synthesis of a library of new steroids with potential contraceptive properties. These methods can be employed in the production of such compounds with high stereoselectivity².

The biological transformation of ethynodiol diacetate (81) with *Cunninghamella elegans* and plant cell suspension cultures of *Ocimum basilicum* and *Azadirachta indica* was carried out. Biotransformation of 81 with *Cunninghamella elegans* yielded three new hydroxylated compounds, characterized as 17 α -

ethynylestr-4-en-3 β ,17 β -diacetoxy-6 α -ol (82), 17 β -ethynylestr-4-en-3 β ,17 β -diacetoxy-6 β -ol (83), and 17 α -ethynylestr-4-en-3 β ,17 β -diacetoxy-10 β -ol (84) and 17 α -ethynyl-17 β -acetoxyestr-4-en-3-one (85) as described in Figure 13.

The biotransformation of 81 with *Ocimum basilicum* included hydrolysis of the ester group, oxidation of alcohol into ketone, and rearrangement of the hydroxyl group. Thus four major metabolites were characterized as 17 α -ethynyl-17 β -acetoxyestr-4-en-3-one (85), 17 α -ethynyl-17 β -hydroxyestr-4-en-3-one (86), 17 α -ethynyl-3 β -hydroxy-17 β -acetoxyestr-4-ene (87) and 17 α -ethynyl-5 α ,17 β -dihydroxyestr-3-ene (88). Biotransformation of 81 with *Azadirachta indica* culture yielded compounds 85 and 86 as described in Figure 14².

Exemstane (89: trade name aromasin) is a steroidal irreversible aromatase inhibitor, used for the treatment of breast cancer. Microbial

transformation of anti-cancer steroid, exemestane (89), was investigated by using two filamentous fungi, *Macrophomina phaseolina*, and *Fusarium lini*. Fermentation of 89 with fungi afforded four polar hydroxyl metabolites, 11 α -hydroxy-6-methylene-androsta-1,4-diene-3,17-dione (90), 16 β ,17 β -

dihydroxy-6-methylene-androsta-1,4-diene-3-one (91), and 17 β -hydroxy-6-methylene-androsta-1,4-diene-3,16-dione (92) and 17 β -hydroxy-6-methylene-androsta-1,4-diene-3-one (93) as described in Figure 15 and 16¹⁸.

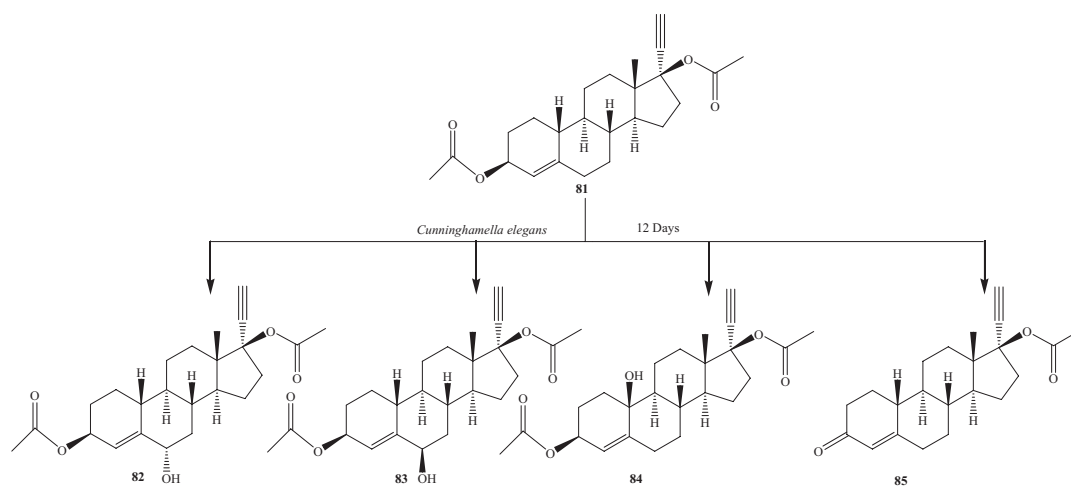


Fig. 13: Biotransformation of ethynodiol diacetate (81) with *Cunninghamella elegans*

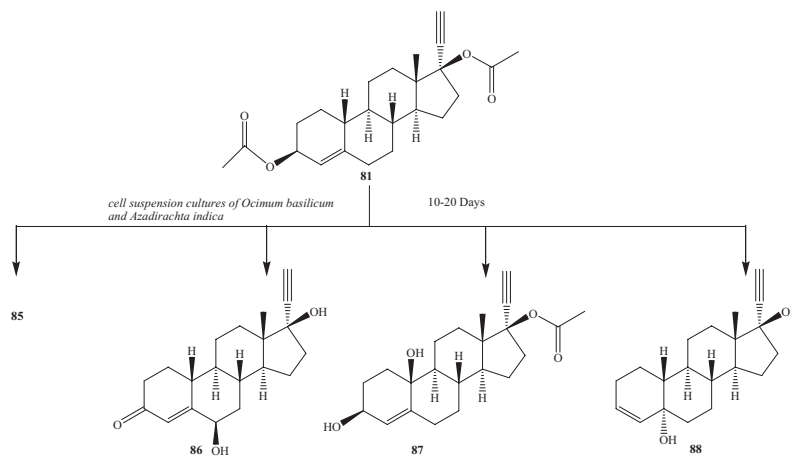


Fig. 14: Biotransformation of ethynodiol diacetate (81) with cell suspension cultures of *Ocimum basilicum* (compounds 85–88, in 20 days) and *Azadirachta indica* (compounds 85 and 86, in 10 days)

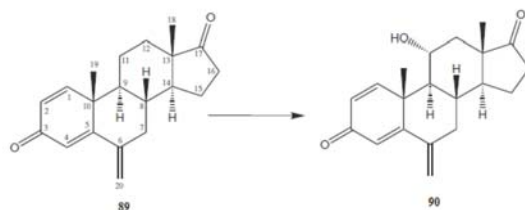


Fig. 15: Biotransformation of exemestane (89) with *Fusarium lini* yielded metabolite 90

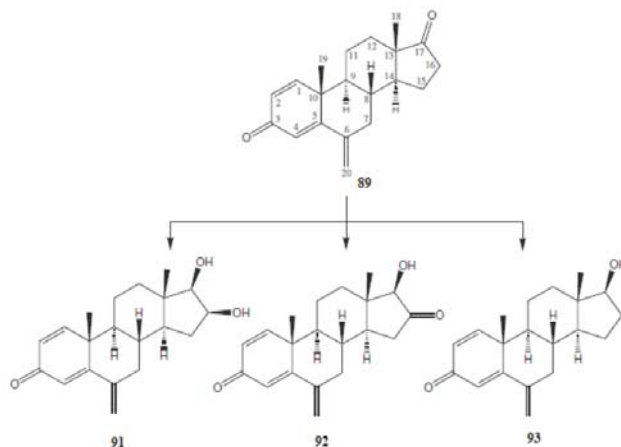


Fig. 16: Biotransformation of exemestane (89) with *Macrophomina phaseolina* yielded metabolites 91-93

Microbial transformation of methylgestrone (94), an oral contraceptive was investigated by using *Macrophomina phaseolina*, *Aspergillus niger*, *Gibberella fujikuroi*, and *Cunninghamella echinulata* produced eleven metabolites 95–105. Metabolites were resulted from the hydroxylation at various positions such as C-1, C-2, C-6, C-10, C-11, and C-17 α -CH₃, as well as aromatization of ring A of the steroidal skeleton of substrate 94. The transformed products were identified as 17 α -methyl-6 β ,17 β -dihydroxyestr-4-en-3-one (95), 17 α -(hydroxymethyl)-11 β ,17 β -

dihydroxyestr-4-en-3-one (96), 17 α -methyl-2 α ,11 β ,17 β -trihydroxyestr-4-en-3-one (97), 17 α -methyl-1 β ,17 β -dihydroxyestr-4-en-3-one (98), 17 α -methyl-11 α ,17 β -dihydroxyestr-4-en-3-one (99), 17 α -methyl-11 β ,17 β dihydroxyestr-4-en-3-one (100), 17 α -methyl-10 β ,17 β -dihydroxyestr-4-en-3-one (101), 17 α -(hydroxymethyl)-17 β -hydroxyestr-4-en-3-one (102), 17 α -methylestr-1,3,5(10)-trien-3,17b-diol (103), 17 α -methyl-3,17 β -dihydroxyestr-1,3,5(10)-trien-6-one (104), and 17 α -methyl 6 β ,10 β ,17 β -trihydroxyestr- 4-en-3-one (105) as described in Figure 17, 18, 19 and 20¹⁹.

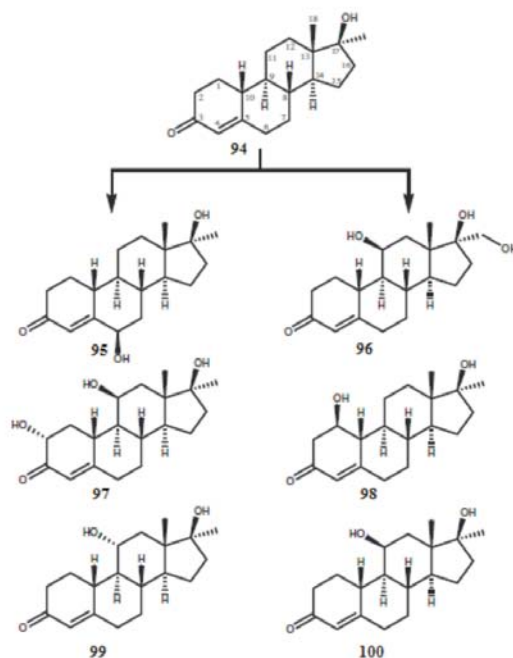


Fig. 17: Biotransformation of methylgestrone (94) with *Macrophomina phaseolina*

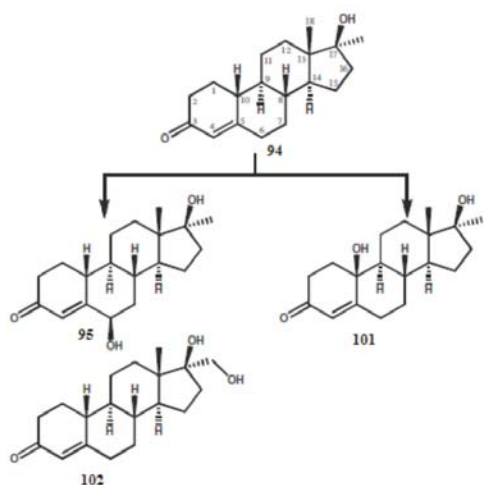


Fig. 18: Biotransformation of methyloestrenolone (94) with *Aspergillus niger*

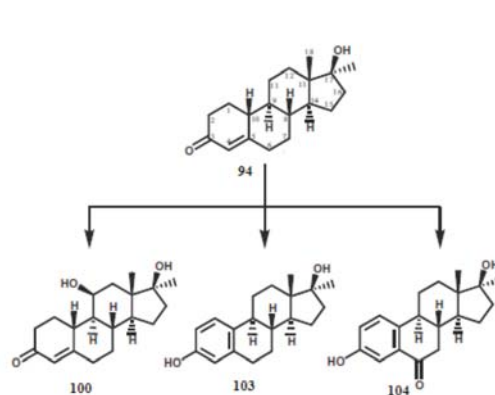


Fig. 19: Biotransformation of methyloestrenolone (94) with *Gibberella fujikuroi*

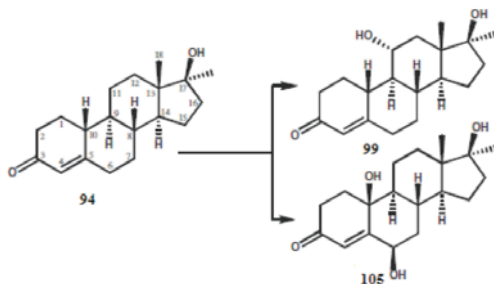


Fig. 20: Biotransformation of methyloestrenolone (94) with *Cunninghamella echinulata*

Biotransformation of anabolic steroidal drugs

The term anabolic steroid means any drug or hormonal substance, chemically and pharmacologically related to testosterone (other than estrogens, progestins and corticosteroids), that promotes muscle building. Anabolic-androgenic steroids were first developed in the 1930s to promote the growth of skeletal muscles and to develop male sexual characteristics. They were also used medicinally for the treatment of anemia and osteoporosis. Ethylestrenol (106) is an anabolic steroid, containing a 17-alkyl group with some progestational activity and androgenic effects. It is widely used for the treatment of leucopenia, Sjogren's syndrome-like disorders and hemophilia B Leyden. Ethylestrenol (106) was fermented with

the plant pathogenic fungus *Rhizopus stolonifer* afforded 17 α -ethyl-3 β ,17 β -dihydroxy-19-norandrost-4-ene (107) and 17 α -ethyl-17 β -hydroxy-19-norandrost-4-en-3-one (108) as shown in Figure 21²⁰.

Nandrolone (109) is a natural anabolic steroid which occurs in the human body in tiny quantities and it is used for the treatment of autoimmune hemolytic anemia. Nandrolone (109) when incubated with *Rhizopus stolonifer*, yielded two oxidative metabolites, 19-norandrost-4-en-3,17-dione (110) and 6 α ,17 β -dihydroxy-19-norandrost-1,4-dien-3-one (111) as shown in Figure 22²⁰.

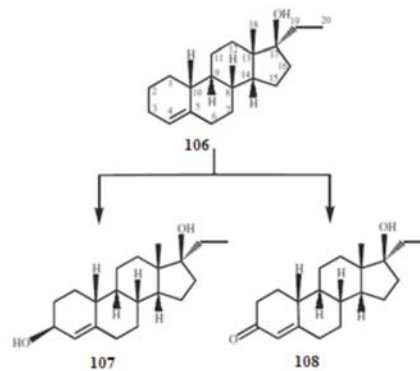


Fig. 21: Biotransformations of Ethylestrenol (106) by *Rhizopus stolonifer*

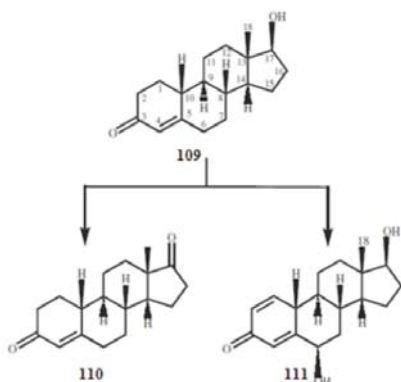


Fig. 22: Biotransformations of Nandrolone (109) by *Rhizopus stolonifer*

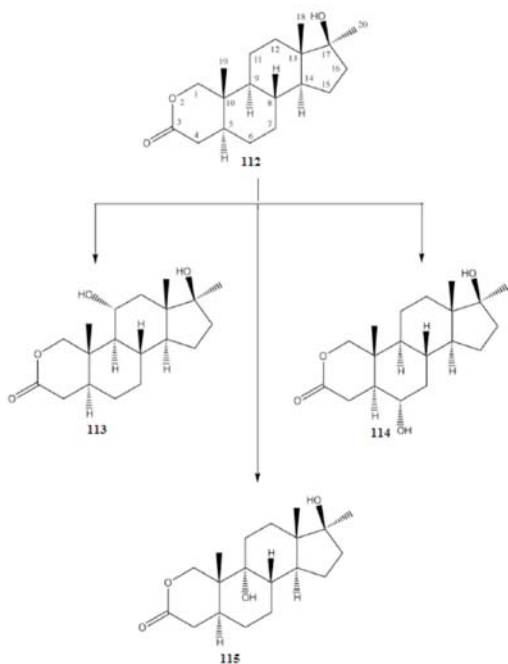


Fig. 23: Biotransformation of oxandrolone (112) with *Rhizopus stolonifer*

Biotransformation of an anabolic synthetic derivative of testosterone, oxandrolone (112), by using suspended-cell cultures of the plant pathogen fungus *Rhizopus stolonifer*, resulted in the production of three hydroxyl metabolites. These metabolites were identified as 11a-hydroxyoxandrolone (113), 6a-hydroxyoxandrolone (114) and 9a-hydroxyoxandrolone (115) as shown in Figure 23. Metabolite 112 and 114 showed significant β -glucuronidase inhibitory activity²¹.

CONCLUSION

In conclusion, the current trends in microbial and plant cell-based catalysis of steroids make it possible to predict revolutionary achievements in steroid industry based on "whole-cell based factories" for production of steroid hormones. The advantage in this field is to be focused on "green" solvents and to turn from the two-phase water/organic systems towards more ecologically friendly media. The microbial technology process also provides accesses to regions of the steroidal skeletons, which are difficult to be functionalized by conventional chemical methods. Moreover, laboratory results are characterized as potentially interesting for further industrial applications, but can hardly be used in industrial practice because a cost-efficient industrial process requires a productivity of at least few grams per liter. In order to improve biotransformation processes, problems of low steroid solubility, insufficient substrate availability, and, in some cases, toxicity of substrate/product to microbial cells should be solved.

ACKNOWLEDGMENTS

Author was funded by the Fundamental Research Grant Scheme (FRGS) (600-RMI/FRGS 5/3, 12/2012), Ministry of Higher Education, Malaysia.

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