Chapter

Biotransformation of Steroids Using Different Microorganisms

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Abstract

The introduction of a hydroxyl group "biohydroxylation" in the steroid skeleton is an important step in the synthesis of new steroids used physiologically as hormones and active drugs. There are currently about 300 known steroid drugs whose production constitutes the second category within the pharmaceutical market after antibiotics. Several biotransformations at industrial scale have been applied in the production of steroid hormones and drugs, which have functionalized different types of raw materials by means of *chemo*-, *regio*-, and *stereo*selective reactions (hydroxylation, Baeyer-Villiger oxidation, oxidation reactions, reduction of group carbonyl, isomerization, and Michael additions, condensation reactions, among others). In Green Chemistry, biotransformations are an important chemical methodology toward more sustainable industrial processes.

Keywords: biotransformation, steroid compounds, biological transformation, bioconversions, microorganisms

1. Introduction

Steroids (stereos = solids) are organic compounds derived from alcohols, which are widely distributed in the animal and plant kingdoms. Their base skeleton has 17 carbon atoms in a tetracyclic ring system known as cyclopentanoperhydrophenanthrenes (gonane and estrane). In this group of substances, life-vital compounds are categorized, such as cholesterol, bile acids, sex hormones, vitamin D, corticosteroids, cardiac aglycones, and antibiotics, among others.

Some of the most potent toxins are steroid alkaloids. Steroids are responsible for important biological functions in the cell; for example, the steroids derived from androstane, pregnane, and estrane have hormonal activity [1–5]; bile acids are important for the digestion and absorption of fats; and cardiotonic aglycones are used for the treatment of heart disease. Sterols are constituents of the cell membrane, essential for cell stability and development; also, they are precursors of bile acids and steroid hormones.

A large number of steroids are used as anti-inflammatory agents [6], immunosuppressants, progestational agents, diuretics, anabolics, and contraceptives [7–9]. Some are used for the treatment of prostate and breast cancer [10, 11], for adrenal insufficiency [12], for prevention of heart disease [13], as antifungal agents [14], and as active ingredients used for the treatment of obesity [15] and AIDS [16]. Recently, the antiviral activity against the herpes simplex virus type I of some steroid glycosides was determined [17].

The therapeutic action of some steroid hormones has been associated with their interaction with intracellular receptors, which act as transcription factors in the regulation of gene expression [18]. It has been reported that some steroids, such as dehydroepiandrosterone (DHEA), progesterone, pregnenolone and its sulfated derivatives [19, 20], as well as, 17β-estradiol, allopregnanolone and its synthetic derivatives (afoxolaner and ganaxolone) are considered neurosteroids, due to their action at the level of the CNS [19].

The physiological activity of steroids depends on their structure, the type, number, spatial orientation, and reactivity of the different functional groups present in the tetracyclic core as well as the oxidation state of the rings. For example, the presence of an oxygenated function in C-11β is crucial for the anti-inflammatory activity; the hydroxyl function in C-17β determines androgenic properties; the aromatization of ring A confers estrogenic effect; and corticosteroids have the 3-keto-4-ene group and the pregnane side chain at C-17 [21, 22].

Currently, about 300 steroid drugs are known, and this number tends to grow. Their production represents the second category in the pharmaceutical market after antibiotics [24, 25]. Nowadays, steroids represent one of the largest sectors in pharmaceutical industry with world markets in the region of US$ 10 billion and the production exceeding 1,000,000 tons per year [23].

The production of steroid drugs and hormones is one of the best examples of the applications that biotransformations have on an industrial scale [3, 21]. Microbiological transformations are an effective tool for the preparation of various compounds [26], which can be difficult to obtain by conventional chemical methods and have been widely used in the bioconversion of steroids [25]. In 1950, the pharmacological effects of cortisol and progesterone were reported, in addition to the hydroxylation of the latter in C-11α using *Rhizopus* species. This began a very important stage in the development of the synthesis of steroids with biological activity [4, 5].

Currently, a great versatility of microbial systems in the pharmaceutical industry for the commercial production of steroids and other drugs is recognized [27, 28]. Several hundreds of microbiological transformations of steroids have been reported in the literature; also, many bioconversions have been incorporated into numerous partial syntheses of new compounds for their evaluation such as hormones or drugs [21, 29–32]. Chemical derivatives of some steroids are reported to have better therapeutic advantages than the starting materials.

However, the main objectives in the research and development of the steroid drug industry currently consist of the detection and isolation of microbial strains with novel activity or more efficient transformation capacity, where genetic engineering and metabolic engineering can play a prominent role in the metabolism of bacteria, fungi, and plants [33–36].

The aim of the present review is to emphasize the importance of biotransformation using microorganisms to obtain steroid compounds with pharmaceutical interest, as a chemical-biological strategy that alternates with the chemical synthesis, and to highlight the chemical reaction made by different types of microorganisms in the functionalization of the steroid skeleton.

2. Microbiological transformations of steroids

In Green Chemistry, biotransformations constitute an important methodology in organic chemistry [37]. The microbiological transformations of steroids have been an essential chemical tool used for the preparation of many intermediaries and in the generation of new drugs, where chemical functionalization-hydroxylation, Baeyer-Villiger oxidation, reduction, isomerization, Michael additions, and condensation reactions can be carried out in different positions of the steroid skeleton in *chemo-*, *regio-*, and *stereo*selective ways, being very complicated or even impossible by the classic chemical methods. Currently, any stereogenic center of the steroid skeleton can be specifically hydroxylated stereoselectively. Nowadays, *biohydroxylations* in C-11α, 11β, 15α, and 16α are industrially carried out via a microbial hydroxylation with good yields and enantiomeric excess (ee). Below are some of the microbiological transformations performed on different natural and synthetic steroids [25].

In the literature, it is the well-documented *regio-* and *stereo*selective hydroxylation in C-14 with α orientation in progesterone (**1**) and other steroids by well-functioning fungi, such as *Thamnostylum piriforme* (ATCC 8992), *Mucor griseocyanus* (ATCC 1207a), *Actinomucor elegans* (MMP 3132), and *Zygodesmus* sp. (ATCC 14716).

From the incubation of **1** with *T. piriforme,* 14α-hydroxyprogesterone (**2**, 32%) and 9α-hydroxyprogesterone (**3**, 1.4%) were obtained; whereas in the incubation of **1** with *M. griseocyanus,* **2** (13.4%), 7α,14α-dihydroxyprogesterone (**4**, 6.5%) and 6β,14α-dihydroxyprogesterone (**5**, 2.8%) were obtained. In the biotransformation of **1** using *A. fumigatus* after 24 h of incubation, different mono-and dihydroxylated products were obtained: 11α-hydroxyprogesterone (**6**, 33%), 11α,15β-dihydroxyprogesterone (**7**, 17%), 7β,15β-dihydroxyprogesterone (**8**, 14%), 15β-hydroxyprogesterone (**9**), 7β-hydroxyprogesterone (**10**), where **9** and **10** were detected in minimal quantity. Finally, at 72 h, the main products were 7 (48%) and 8 (25%), with the positions 11α and 15β being hydroxylated more easily than the position 7β in **1** [38, 39].

In the incubation of **1** with *Saprolegnia hypogyna*, 4-androstene-3,17-dione (**11**), testosterone (**12**), and testolactone (**13**) were obtained [40]. The compounds **13** (98%) were also obtained from the bioconversion of **1** using *A. sojae* (PTCC 5196). The biotransformation pathway indicating the presence of Baeyer-Villiger monooxygenase (BVMO) can carry out both oxygenative esterification of 20-ketosteroids and oxygenative lactonization of 17-ketosteroids [41]. The compounds 15α-hydroxyprogesterone (**14**, 47%) and 12β,15α-dihydroxyprogesterone (**15**, 25%) were isolated in the biotransformation of **1** using *Fusarium culmorum* [42]. In the biotransformation of **1** using the bacterium, thermophilic *Bacillus stearothermophilus*, four products of monohydroxylation, 20α-hydroxyprogesterone (**16**, 61%), 6β-hydroxyprogesterone (**17**, 21%) and 6α-hydroxyprogesterone (**18**, 14%), and 9,10-seco-pregnen-3,9,20-trione (**19**, 4%), were isolated [43].

An efficient *regio*- and stereoselectivity was observed in the biotransformation of **1** on a large scale by the system *Mucor* 881 (M881) to give the hydroxylated derivatives **6**, 6β,11α-dihydroxyprogesterone (**20**), and 6β-hydroxypregn-4-ene-3,11,20-trione (**21**). In the literature, it is described that species of the genus *Mucor* and *Rhizopus* can hydroxylate said positions but with lower yields. The fungal system M881 showed the ability to carry out hydroxylation at 6β and 11α positions of 4-ene-3-one steroids (**1**, **11**, **12** and **211**) [44].

Recently, it was reported that in the biotransformation of **1** using *Penicillium aurantiogriseum* for 10 days, **11** and androsta-1.4-dien-3,17-dione (**22**) were obtained. These products were observed in the biotransformation of **1** using *Bacillus sphaericus*; the hydroxylation in C-17 was mainly observed [45, 46]. Biotransformation of **1** using *Geobacillus gargensis* (DSM 15378) has resulted in the production of secoderivatives: **19** and **23** (9,10-seco-4-pregnene-20α-hydroxy-3,9-dione), which are produced by the rupture of the ring B of **1** (**Figure 1**) [47]. Secosteroids are an important group, which exhibits a variety of different biological activities [48, 49].

In the biotransformation of 5β-dihydroprogesterone (**24**) using *T. piriformis,* 14α-hydroxy-5β-pregnan-3,20-dione (**25**, 11.8%), 3β,14α-dihydroxy-5β-pregnan-20-one (**26**, 0.5%), and 14α,15β-dihydroxy-5β-pregnan-3,20-dione (**27**, 0.4%) were characterized, while in the biotransformation of 3β-hydroxy-5β-pregnan-20-one (**28**), **26**

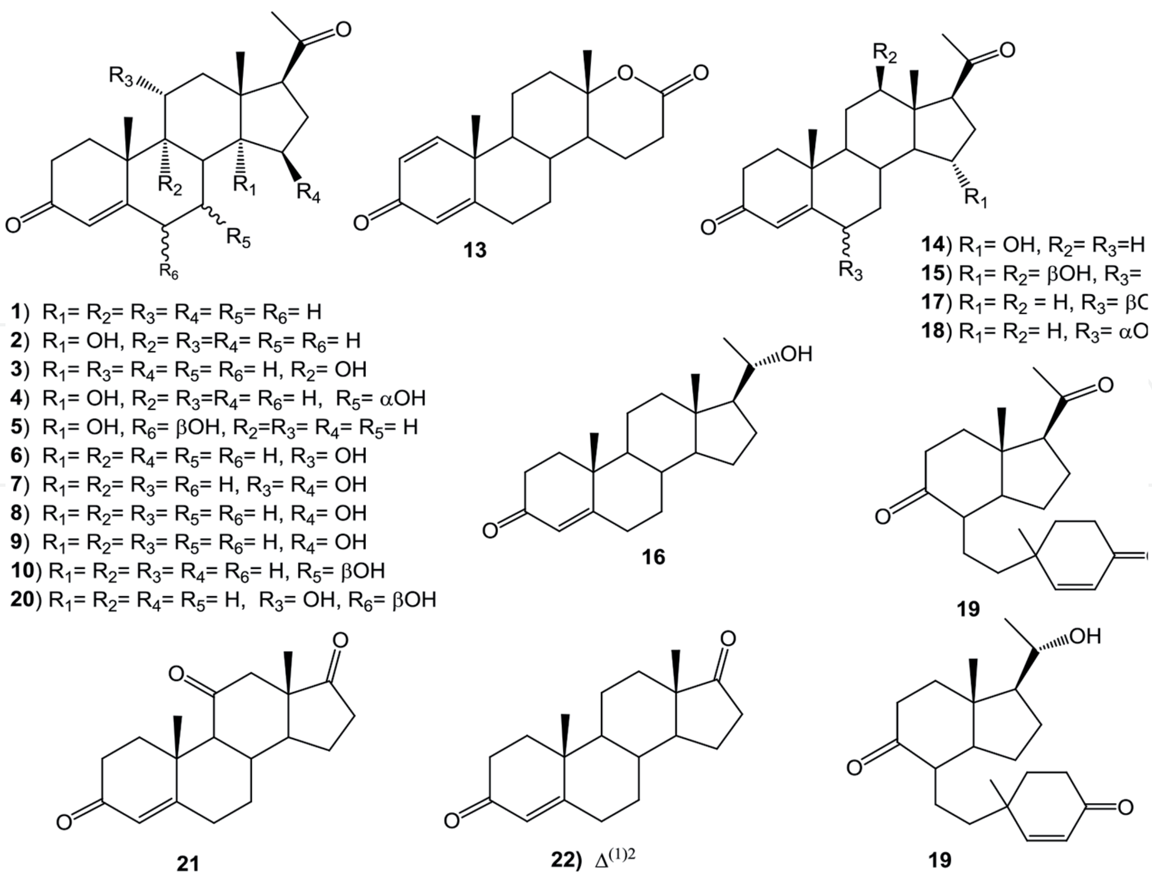


Figure 1.
Biotransformation products of progesterone (1).

(0.6%) and 3β,9α,14α-trihydroxy-5β-pregnan-20-one (**29**, 16%) were isolated, after being incubated for 96 h. The microbiological transformation of **28** using *Actinomucor elegans* produced the compounds **25** and **28** in lower yield than *T. piriforme* and a minor product identified as 3β,9α-dihydroxy-5α-pregnan-20-one (**30**) (**Figure 2**) [38].

The biotransformation of 16-dehydroprogesterone (4,16-pregnadien-3,20-dione, **31**) using *Mucor piriformis* has been reported to give different hydroxylation products: 14α-hydroxypregna-4,16-dien-3,20-dione (**32**, 1%), 7α,14α-dihydroxypregna-4,16-dien-3,20-dione (**33**, 78%), 3β,7α,14α-trihydroxy-5α-pregna-16-en-20-one (**34**, 3%), and 3α,7α,14α-trihydroxy-5α-pregna-16-en-20-one (**35**, 2%); while the microsomes

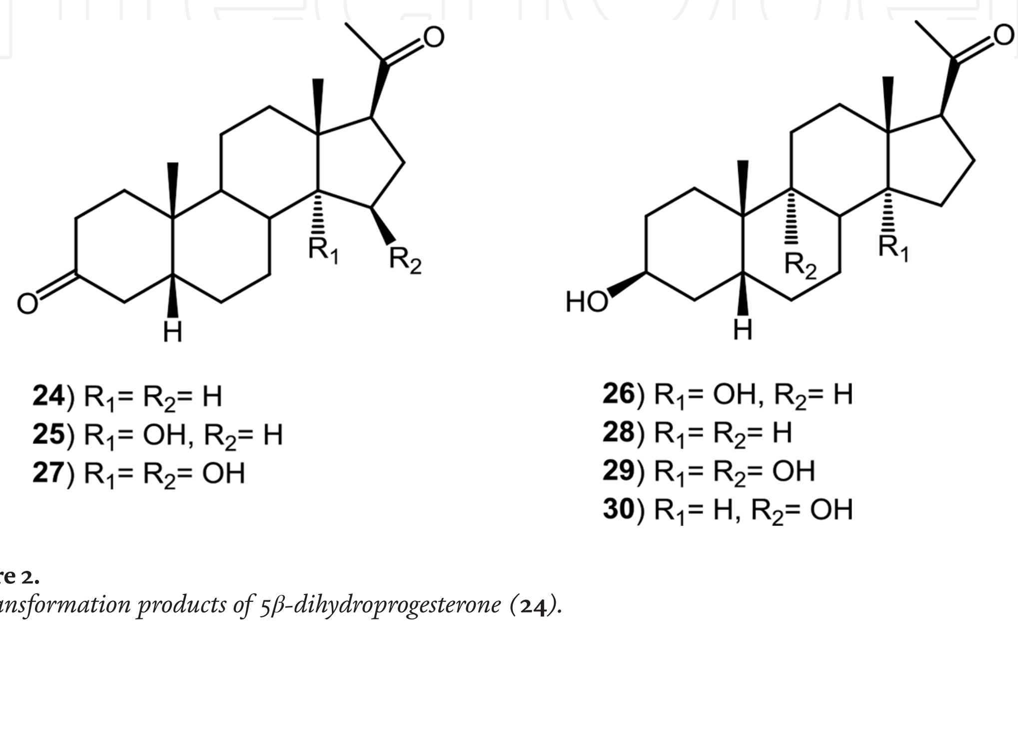


Figure 2.
Biotransformation products of 5β-dihydroprogesterone (24).

prepared from **31** transformed the hydroxylate to 14α-hydroxy derivative (**32**). Incubation of **32** with *M. piriformis* resulted in the formation of **33–35** (**Figure 3**) [50].

In contrast, in the biotransformation of 17α-hydroxyprogesterone (**36**) using *M. piriformis*, after 48 h of incubation, four compounds were obtained: 17α,20α-dihydroxypregn-4-en-3-one (**37**, 19%), 7α,17α-dihydroxypregn-4-en-3,20-dione (**38**, 25%), 6β,17α,20α-trihydroxy-pregn-4-en-3-one (**39**, 18%), and 11α,17α,20α-trihydroxypregn-4-en-3-one (**40**, 25%); it was observed that *M. piriformis* was able to hydroxylate the C-6, C-7, C-11, and C-14 positions stereospecifically, in addition to reducing the 4-en-3-one system in ring A and the keto group of C-20 (**Figure 4**) [50]. The biotransformation of **36** using *Fusarium culmorum* led to the formation of **14** (47%) and **15** (25%) [42].

Pregnenolone (3β-hydroxypregn-5-en-20-one, **41**), the precursor of many steroid hormones, was biotransformed by *Mucor piriformis* to obtain two metabolites, 3β,7α-dihydroxypregn-5-en-20-one (**42**) and 3β,7α,11α-trihydroxypregn-5-en-20-one (**43**) [51], where **43** (46.4%) was also a bioconversion product of **41** using *Mucor circinelloides* var. *lusitanicus* [52]. Two metabolites of pregnenolone (**41**) obtained from biotransformation of *B. cinereae* were characterized as 3β,11α,16β-trihydroxypregn-5-en-20-one (**44**, 39%) and 11α,16β-dihydroxypregn-4-en-3,20-dione (**45**, 6%). The formation of the hydroxylation products in C-11 and C-16 by *B. cinereae* can be determined by the presence of the acetyl group in C-20 [53]. The biotransformation of **41** using different microorganisms (*Cunninghamella elegans*, *R. stolonifer*, and *G. fujikuroi*) was reported by Choudhary et al. [54]. Incubation of **41** with *C. elegans* produced 3β,7β,11α-trihydroxypregn-5-en-20-one (**46**, 28%), 3β,6α,11α,12β,15β-pentahydroxypregn-4-en-20-one (**47**, 4%), and 3β,6β,11α-trihydroxypregn-4-en-20-one (**48**, 2%), while incubation with *G. fujikuroi,* two products 3β,7β-dihydroxypregn-5-en-20-one (**49**, 3%) and 6β,15β-dihydroxypregn-4-en-3,20-dione (**50**, 2%) were obtained. In the microbiological transformation of **41** using different *Bacillus* strains, **42**, **49**, and 7-oxo-pregnenolone (**51**) were the major products obtained [55], while by using *Fusarium oxysporum* var. *cubense,* **42** was the only product obtained [56]. The biotransformation of pregnenolone acetate (**52**) using *C. elegans* generated **41**, **22**, 6β,15β-dihydroxyandrosta-4-en-3,17-dione (**53**), and 11α,15β-dihydroxypregn-4-en-3,20-dione (**54**), while by using *R. stolonifer*, 11α-hydroxypregn-4-en-3,20-dione (**55**) and **53** were obtained (**Figure5**) [54].

The microbiological transformation of the racemic mixture of 13-ethyl-17β-hydroxy-18,19-dinor-17α-pregn-4-en-20-yn-3-one (**56**) was tested with different fungi *Rhizopus nigricans*, *R. arrhizus*, *Aspergillus niger*, *A. ochraceus*, and *Curvularia lunata.* The bioconversion of the racemic mixture of **53** by *R. arrhizus* produced only one major product, (±)-13-ethyl-10β,17β-dihydroxy-18,19-dinor-17α-pregn-4-en-20-yn-3-one

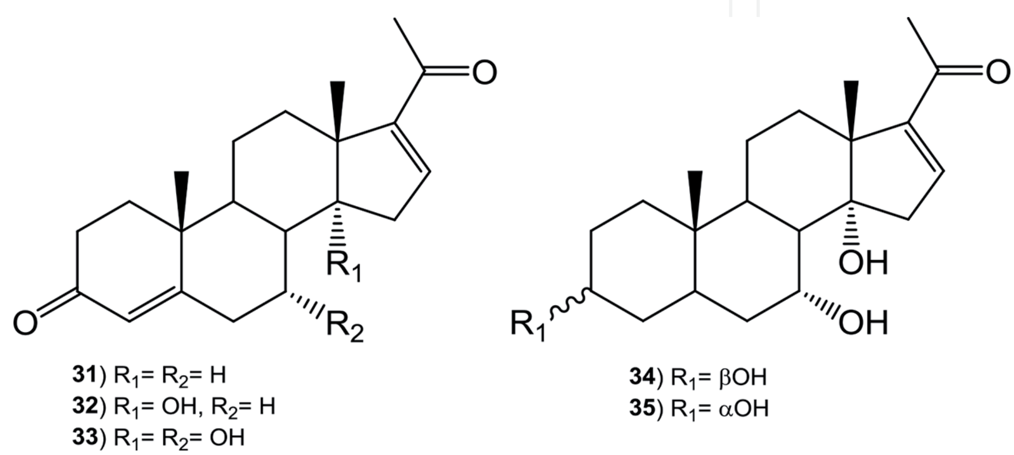
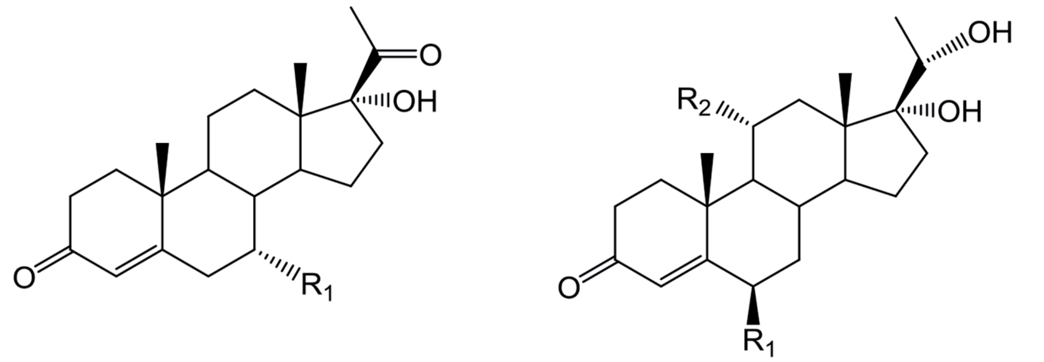


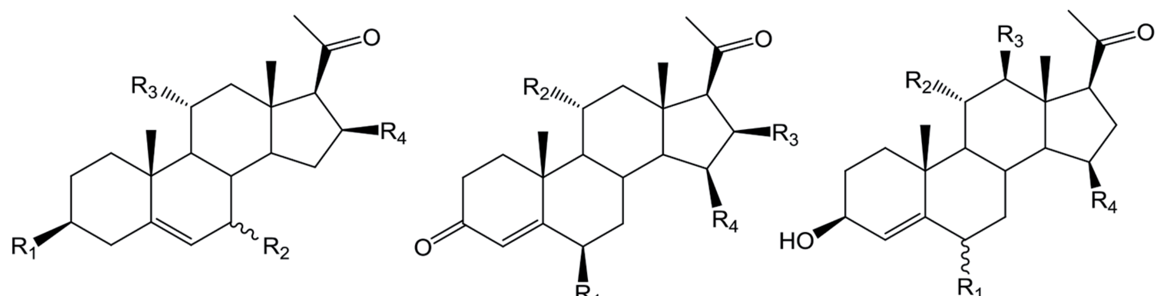
Figure 3.
Biotransformation products of 16-dehydroprogesterone (31).



36) R_1= H
38) R_1= OH

37) R_1=R_2= H
39) R_1=OH R_2= H
40) R_1= H, R_2= OH

Figure 4.
*Biotransformation products of 17α-hydroxyprogesterone (**36**).*



41) R_1= OH, R_2= R_3=R_4= H
42) R_1= OH, R_2= αOH, R_3= R_4= H
43) R_1= R_3= OH, R_2= αOH, R_4= H
44) R_1= R_3= OH, R_2= αOH, R_4= H
46) R_1= OH, R_2= βOH R_3= R_4= H
49) R_1= OH, R_2= βOH, R_3= R_4= H
51) R_1= OH, R_2= R_3= R_4= H
52) R_1= Ac, R_2= R_3= R_4= H

45) R_1= R_4= H, R_2= R_3= OH
50) R_1= R_4= OH, R_2= R_3= H
53) R_1= R_4= OH, R_2= R_3= H
54) R_1= R_3= H, R_2= R_4= OH
55) R_1= R_3= R_4= H, R_2= O H

47) R_1= αOH, R_2= R_3= R_4= OH
48) R_1= βOH, R_2= OH, R_3= R_4= H

Figure 5.
*Biotransformation products of pregnenolone (**41**) and acetyl derivate (**52**).*

(**57**, 28.4%), whereas *R. nigricans*, *A. niger*, and *C. lunata* biotransformed **56** to **57** more slowly and inefficiently [57].

The racemic mixture (±)-13-ethyl-7β,17β-dihydroxy-18,19-dinor-17α-pregn-4-en-20-yn-3-one (**58**, 4.3%) was obtained as product of incubating mixture **56** with *A. ochraceus*; none of the fungi tested were able to differentiate the two enantiomers of **56** in the course of the hydroxylation reaction; in addition, the absence of the hydroxylated derivative in C-11 is due to the presence of the ethyl group in C-13 or the ethynyl group in C-17 [57]. The microbiological transformation of the racemic mixture and the dextro enantiomer of compound **56** has been described using different species of *Cunninghamella* [58]. For example, the transformation of the racemic mixture of **56** by *C. blakesleeana* (AS 3.910) produced **57** (5.3%), 13-ethyl-6β,17β-dihydroxy-18,19-dinor-17α-pregn-4-en-20-yn-3-one (**59**, 3.6%), 13-ethyl-15α,17β-dihydroxy-18,19-dinor-17α-pregn-4-en-20-yn-3-one (**60**, 3.0%), and 13-ethyl-6β,10β,17β-trihydroxy-18,19-dinor-17α-pregn-4-en-20-yn-3-one (**61**, 3.6%), while by using *C. echinulata* (AS 3.1990), **61** (3.2%), **57** (1.2%), and enantiomer dextro of **58** (2.9%) were obtained. The transformation of the enantiomer dextro of **56** using *C. blakesleeana* produced **57** (1.2%), **58** (2.9%), and **61** (3.2%), by using *C. echinulata*, the same compounds were obtained but in lower yield. Therefore, the microbial transformation of the racemic mixture and the *d*-enantiomer of **56** using different *Cunninghamella* species gave poor yields and poor resolutions, which were obtained for the hydroxylation reaction (**Figure 6**) [58].

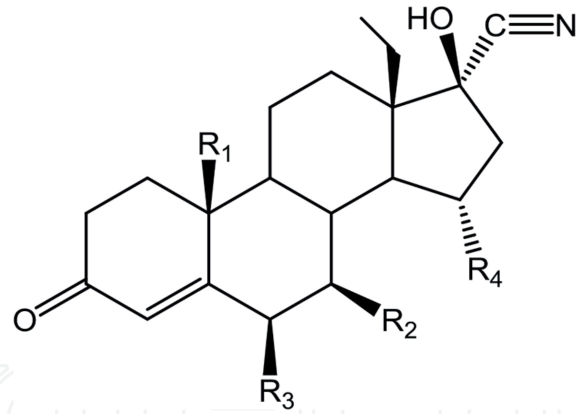


Figure 6.
*Biotransformation products of (+)-13-ethyl-17β-hydroxy-18, 19-dinor-17α-pregn-4-en-20-yn-3-ona (**56**).*

The biotransformation of danazol (17β-hydroxy-17α-pregna-2,4-dien-20-yno-[2,3-d]-isoxazole, **62**), a heterocyclic steroid drug in which an isoxazole ring is fused with ring-A of a steroid nucleus, using *Fusarium lini*, *A. niger* and *Cephalosporium aphidicola* yielded 17β-hydroxy-2-(hydroxymethyl)-17α-pregn-4-en-20-yn-3-one (**63**) and 17β-hydroxy-2-(hydroxymethyl)-17α-pregn-1,4-dien-20-yn-3-one (**64**); while *Bacillus cereus* afforded **64**, as the only product [59]. Microbial transformation of danazol (**62**) using *C. blakesleeana* yielded four compounds: 14β,17β-dihydroxy-2-(hydroxymethyl)-17α-pregn-4-en-20-yn-3-one (**65**, 1.2%), 1α,17β-dihydroxy-17α-pregna-2,4-dien-20-yno-[2,3-d]-isoxazole (**66**, 1.2%), and 6β,7β-dihydroxy-17α-pregna-2,4-dien-20-yno-[2,3-d]-isoxazole (**67**, 0.8%) and **64** (1.2%). This involves hydroxilations al C-1, C-6 and C-15, whereas oxidation at C-3, and N-O bond cleavage has also occurred (**Figure 7**) [60].

Norethisterone (17α-ethynyl-19-nortesterone, **68**) is a potent progestin used as a contraceptive agent; its biotransformation with *Cephalosporium aphidicola* (IMI 68689) produced the aromatization of ring A that yielded 17α-ethynylestradiol (**69**), whereas **69** was biotransformed by *Cunninghamella elegans* (NRRL 1392) producing the compounds 19-nor-17α-pregna-1,3,5(10)-trien-20-yn-3,4,17β-triol (**70**), 19-nor-17α-pregna-1,3,5(10)-trien-20-yn-3,7α,17β-triol (**71**), 19-nor-17α-pregna-1,3,5(10)-trien-20-yn-3,11α,17β-triol (**72**), 19-nor-17α-pregna-1,3,5(10)-trien-20-yn-3,6β,17β-triol (**73**), and 19-nor-17α-pregna-1,3,5(10)-trien-20-yn-3,17β-diol-6β-methoxy (**74**) (**Figure 8**) [61].

Mestranol (**75**) and 17β-methoxymestranol (**76**) are the mono- and dialkylated derivatives of **69**, respectively. In incubating **75** with *C. elegans*, two hydroxylated compounds were obtained: 6β-hydroxymestranol (**77**, 2.8%) and 6β,12β-dihydroxymestranol (**78**, 3.6%), inferring that the presence of the methoxyl group in C-3 reduces the number of biotransformation products and introduces hydroxyl groups in C-6 and C-12 with β orientation, while **76** was not biotransformed due to the presence of the methoxyl group in C-17 (**Figure 9**) [62].

Microbial transformation of 6-dehydroprogesterone (**79**) using *A. niger* yielded five metabolites: 6β-chloro-7α,11α-dihydroxypregna-4-en-3,20-dione (**80**, 1.0%), 7α-chloro-6β,11α-dihydroxypregna-4-en-3,20-dione (**81**, 1.33%), 6α,7α,-epoxy-11α-hydroxypregna-4-en-3,20-dione (**82**, 1.33%), 6α,7α,-epoxy-pregna-4-en-3,20-dione (**83**, 2.0%), and 11α-hydroxypregna-4,6-dien-3,20-dione (**84**, 2.33%). Compound 11α-hydroxyandrosta-4,6-dien-3-one (**85**, 15.4%) was obtained through whole cell biotransformation of **79** by *G. fujikuroi* (ATCC 10704). The formation of **80** and **81** is an interesting finding. This route provides an efficient method for the obtention of chlorohydrins from alkene functionality [63]. The compound **84** was obtained through the microbial transformation of **79** using *R. nigricans* [64], *Nigrospora sphaerica*, *Mucor racemosus*, and *Botryosphaeria obtusa*. 6-dehydroprogesterone (**79**)

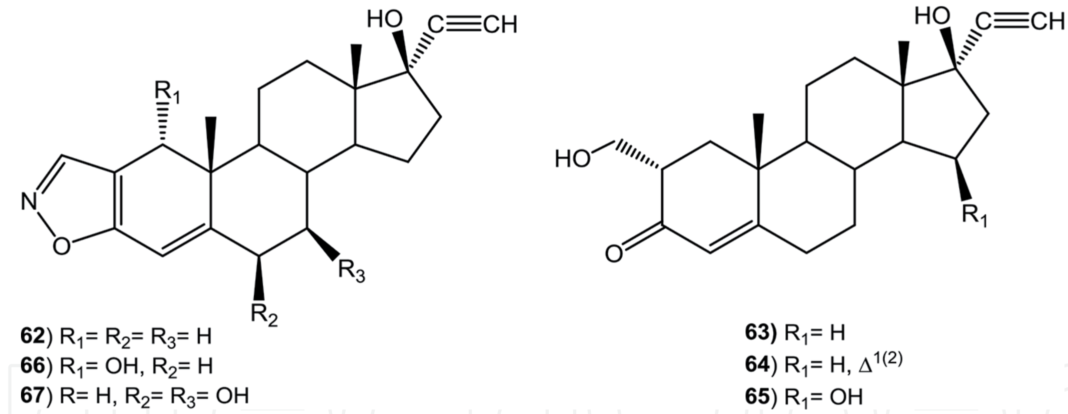


Figure 7.
*Biotransformation products of danzol (**62**)*

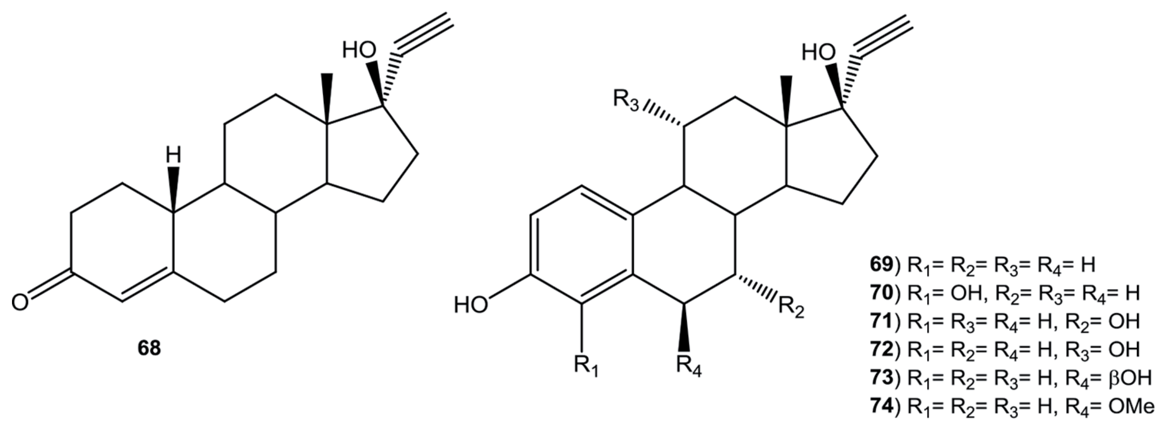


Figure 8.
*Biotransformation products of norethisterone (**68**) and 17α-ethinylestradiol (**69**).*

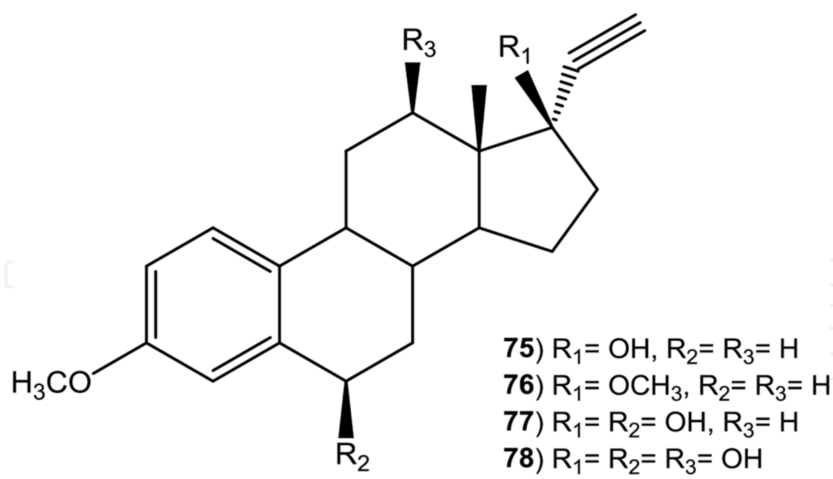


Figure 9.
*Biotransformation of products of mestranol (**75**).*

is a synthetic derivate of progesterone. *Botryodiplodia theobromae* was used for the synthesis of 6-DPH from progesterone (**Figure 10**) [65].

Incubation of melengestrol acetate (**86**) with *C. blakesleeana*, which provides an route for the monohydroxylation of the (**86**) at C-11, yielded a 17α-acetoxy-11β-hydroxy-6-methylenepregna-4,6-diene-3,20-dione (**87**) (**Figure 11**) [66].

Biotransformation of 3β-hydroxy-17β-carboxyethyl-5β-androstenol (**88**) using *T. pyriformis* resulted in the mixture of

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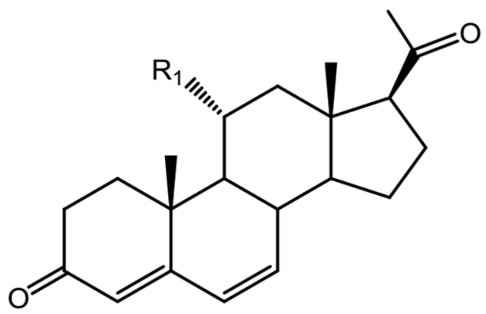
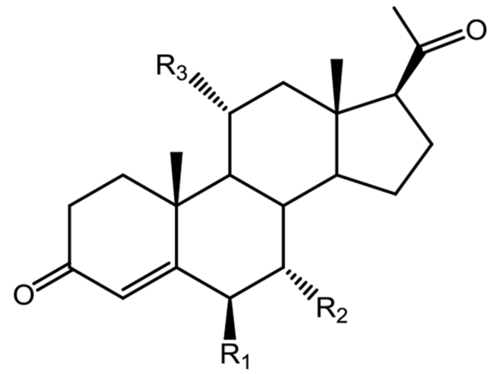
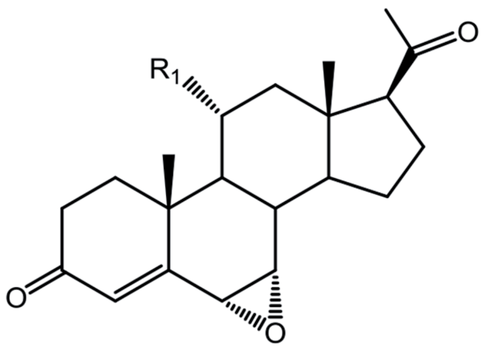


79) R_1= H
84) R_1= OH



80) R_1= Cl, R_2= R_3= OH
81) R_1= R_3= OH, R_2= Cl



82) R_1= OH
83) R_1= H

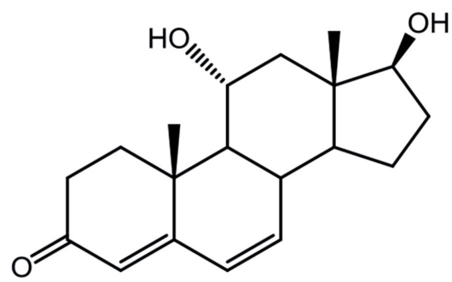


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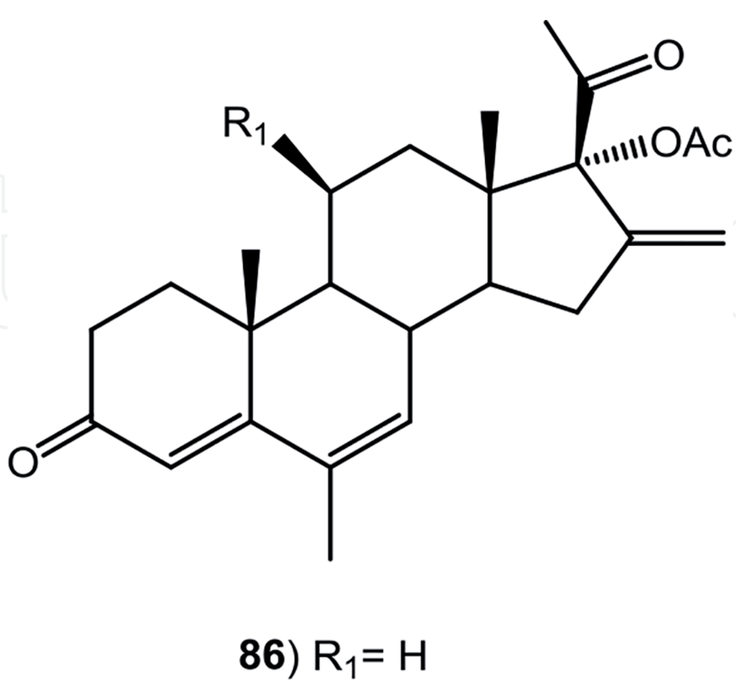
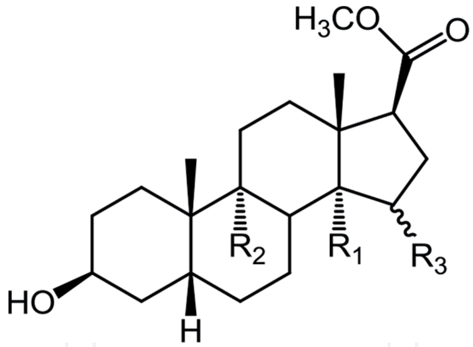
Figure 10.
*Biotransformation products of 6-dehydroprogesterone (**79**).*



86) R_1= H
87) R_1= OH

Figure 11.
*Biotransformation products of melengestrol acetate (**86**).*



88) R_1= R_2= R_3= H
89) R_1= OH, R_2= R_3= H
90) R_1= R_2= OH, R_3= H
91) R_1= OH, R_2= H, R_3= αOH
92) R_1= R_2= H, R_3= βOH

Figure 12.
*Biotransformation products of 3β-17β-carboxyethyl-5β-androsteno (**88**).*

3β,14α-dihydroxy-17β-carboxyethyl-5β-androstenol (**89**, 9%) with 9α,14α-dihydroxy derivative (**90**, 12%) and two minor products 14α,15α-dihydroxy (**91**) and 15β-hydroxy (**92**). Compound **92** was identified as a product of biotransformation using *A. elegans*, *M. griseocyamus*, and *Zygodesmus sp.* (**Figure 12**) [38].

Androst-4-en-3,17-dione (**11**), which plays an important role in the metabolism of drugs, among many other functions, was biotransformed using *M. piriformis* to give one main product, 6β-hydroxyandrost-4-en-3,17-dione (**93**, 13%), and four minor products, 14α-hydroxyandrost-4-en-3,17-dione (**94**, 2%), 7α-hydroxyandrost-4-en-3,17-dione (**95**, 2%), testosterone (**12**, 3%), and 6β-hydroxytestosterone (**96**, 1%). In the biotransformation of **11** using *M. griseocyamus* **94** (9%), **95** (4%) and 14α-hydroxytestosterone (**97**, 9%) were the major products obtained; likewise, **11** and **93** were identified in the mixture of biotransformation products [67]. From the incubation of **11** with *M. piriformis*, **94–97** and 7α,14α-dihydroxytestosterone (**98**) were obtained [38]. Hydroxylated steroids in C-9 are important intermediaries in the synthesis of highly effective anti-inflammatory drugs. The microbiological transformation of **11** to 9α-hydroxyandrost-4-en-3,17-dione (**99**) was studied using *Rhodococcus* sp. in a low-nutrient culture medium at a fixed pH (**Figure 13**) [68]. When **11** was incubated with *Bacillus* strain HA-V6–3, the metabolites **12**, **93–97**,

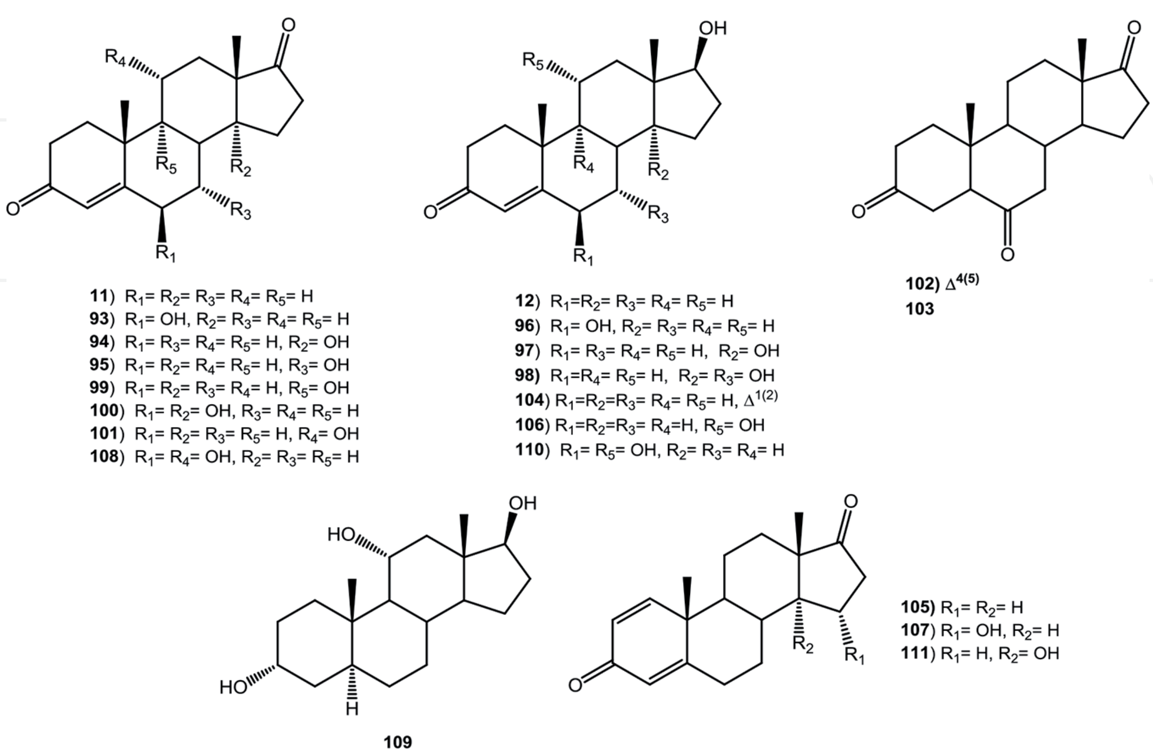


Figure 13.
*Biotransformation products of androst-4-en-3, 17-diona (**11**).*

6β,14α-dihydroxyandrost-4-en-3,17-dione (**100**), 11α-hydroxyandrost-4-en-3,17-dione (**101**), androst-4-en-3,6,17-trione (**102**), and 5α-androst-3,6,17-trione (**103**) were produced as described by Schaaaf and Dettner [69].

In the bioconversion of **11** using *C. aphidicola*, **93** and **94** were obtained [70], while in the fermentation of **11** using *Curvularia lunata,* the products **101** (4%), 17β-hydroxyandrost-1,4-dien-3-one (**104**, 4.4%), androsta-1,4-dien-3,17-dione (**105**, 3%), 11α,17β-dihydroxyandrost-4-en-3-one (**106**, 4%), and **107** (15α-hydroxyandrost-1,4-dien-3,17-dione, 2.8%) were obtained (**Figure 13**) [71]. Biotransformation of **11** using *Beauveria bassiana* was studied in times and with culture media at different pH (pH 6 and 7) [72]. At pH 6, two products were obtained: **106** and 6β,11α-dihydroxyandrost-4-en-3,17-dione (**108**), where the stereoselective hydroxylation was observed at C-11α and C-6β; while at pH 7, the compounds **12**, **106**, 3α,11α,17β-trihydroxy-5α-androstane (**109**), and 6β,11α,17β-trihydroxy-androst-4-en-3-one (**110**) were obtained. Products **93** (14%) and **94** (75%) were isolated from the biotransformation of **11** using *Chaetomium* sp. (**Figure 13**) [73].

Obtaining hydroxylated derivatives in a specific position is one of the objectives of the steroid industry; for example, 14α-hydroxysteroids are shown to have anti-inflammatory, contraceptive, and antitumor activities. With the biotransformation of **11** and **105** using different strains of the fungus, *C. lunata* allowed in the case of **11**, the production of a major product, **94**; while with **105,** 14α-hydroxyandrost-1,4-dien-3,17-dione (**111**, 70%) was obtained (**Figure 13**) [74].

Androsta-1,4-dien-3,17-dione (**105**) is a useful precursor in the chemical or microbiological preparation of other steroid hormones and pharmaceutical. Transformation of **105** by *Colletotrichum lini* (As3.486) produced the hydroxylated compounds at C-11α and C-15α: 15α-hydroxyandrost-1,4-dien-3,17-dione (**107**), 11α,15α-dihydroxyandrost-1,4-dien-3,17-dione (**112**), and 15α,17β-dihydroxyandrost-1,4-dien-3-one (**113**) (**Figure 14**) [75].

Testosterone (**12**) was metabolized by *M. griseocyamus* and *T. piriforme*. In the biotransformation of **12** using *M. griseocyamus*, **97** (35%) and other products were obtained, where **94** was identified as the major product. Conversely, the microbiological transformation of **12** using *T. piriforme* produced **97** (10%), as the main product at 24 h; after 72 h of biotransformation, four products were obtained: **93** (13%), **96** (7%), **97** (13%), and **111** (5%). It was discovered that *T. piriforme* produced smaller quantity of 14α-hydroxy derivatives (**Figure 15**) [38].

In the biotransformation of **12** using *Nectria haematococca*, four substances were isolated, whose performance was dependent on the incubation time; majority of the products were produced at 72 h. The hydroxylated derivatives in C-11 with α

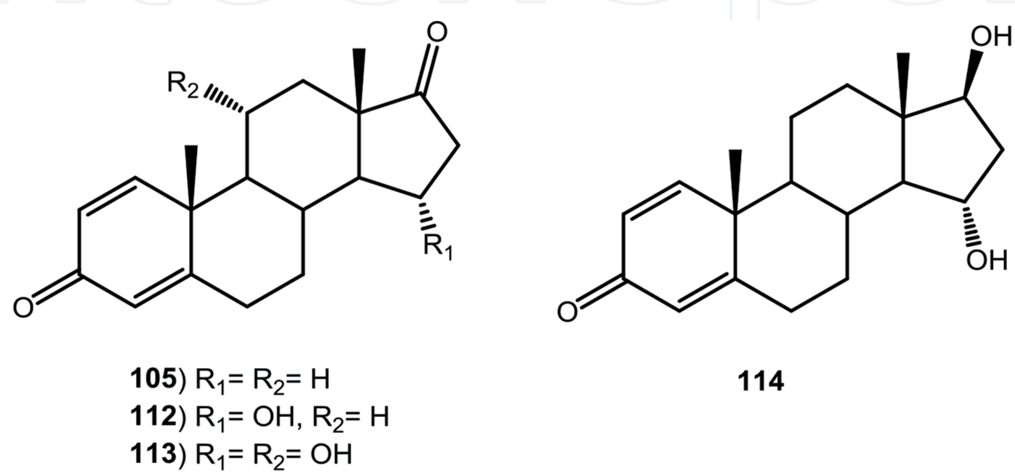


Figure 14.
*Biotransformation products of androsta-1,4-dien-3, 17-dione (**105**).*

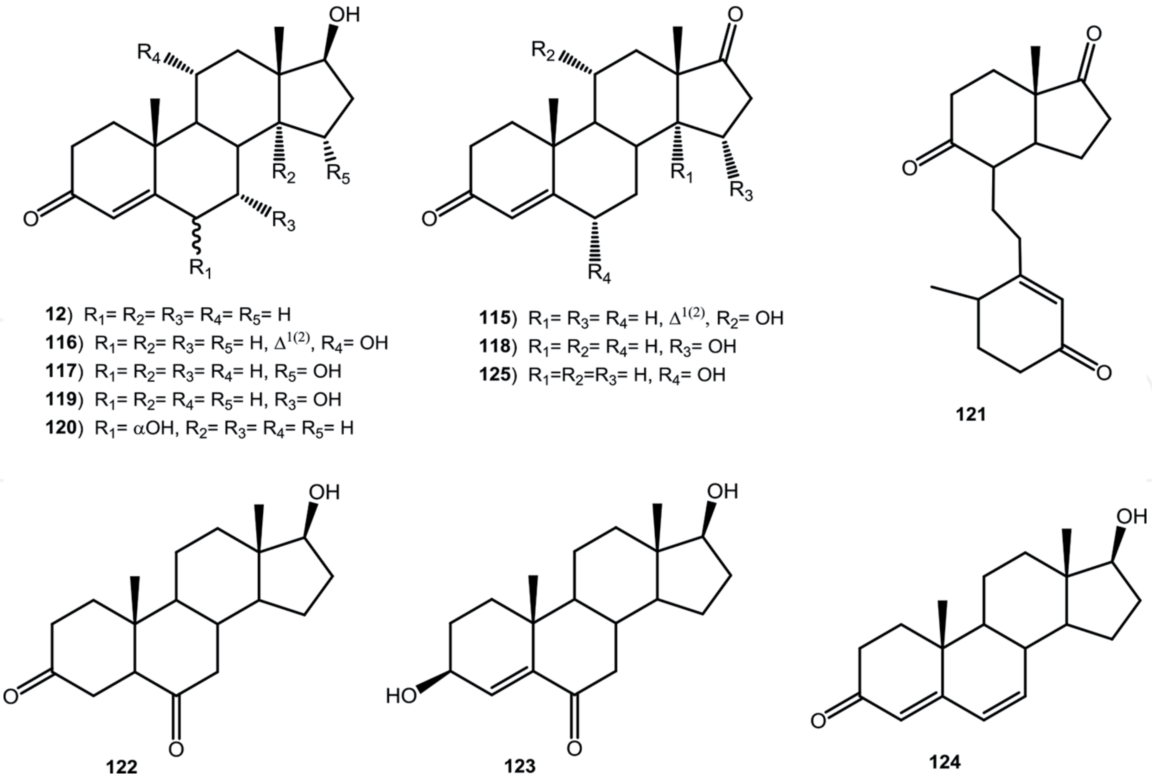


Figure 15.
Biotransformation products of testosterone (12).

orientation and dehydrogenation in C_1-C_2 resulted in the following compounds: 11α-hydroxyandrost-1,4-dien-3,17-dione (**114**, 8.0%), 11α,17β-dihydroxyandrost-1,4-dien-3-one (**115**, 4.3%), **101** (1.9%), and **104** (2.3%) [76]. Incubation of **12** with *Fusarium culmorum* produced **93** (10%) and **96** (32%) with hydroxylated derivatives at C-6β, including the products, 15α,17β-dihydroxyandrost-4-en-3-one (**116**, 22%) and 15α-hydroxyandrost-4-en-3,17-dione (**117**). Selective hydroxylation of **103** at C-6 with a β orientation and allylic position at the unsaturated 3-keto-system is favored by the system π and the presence of the hydroxyl group at C-17, while hydroxylation at C-15 is a very frequent process carried out by fungi of the genus *Fusarium* [42]. Metabolites **11**, **85**, **105,** and **115** were obtained as oxidation and hydroxylation products of **12** using the fungus *F. oxysporum* var. cubense [56]. The fungus, *Cephalosporium aphidicola,* was hydroxylated with **12** to give the products **96** (47%) and **97** (3%), with hydroxylated derivatives in C-6β and C-14α, respectively [70]. Incubation of **12** with *C. lunata* and *Pleurotus ostreatus* yielded compounds **11** (17%) and **115** (13%), respectively [77]. The phytopathogenic fungus, *Botrytis cinerea,* produced 7β,17β-dihydroxyandrost-3-one (**118**, 73%), as the only biotransformation product of **12**. It seems that the presence of the hydroxyl group in C-17 in the androstane skeleton directed the hydroxylation at C-7 with a β orientation (**Figure 15**) [53].

In the biotransformation of **12** using *Bacillus stearothermophilus*, thermophilic bacterium, the major product obtained was **11** (90.2%); it was generated by the oxidation of C-17, and the hydroxylated derivatives of **11** in C-6 (**93**, C-6β, 1.1%) and (**119**, C-6α, 0.9%) include two monohydroxy derivatives of **12**, **96** (C-6β, 3.9%) and **120** (C-6α, 3.9%). This indicates that hydroxylation with α orientation in C-6 may be a common action of some thermophilic bacteria [78]. Biotransformation of **11** using *B. stearothermophilus* in the presence of hydrolase inducers—salicylic acid, chloramphenicol, cyclodextrin, dexamethasone, riboflavin, and rifampicin—resulted in obtaining a higher concentration of the compounds: 9,10-seco-4-androst-3,9,17-trione (**121**), 5α-androst-3,6,17-trione (**103**), 17β-hydroxy-5α-androst-3,6-dione (**122**), 3β,17β-dihydroxyandrost-4-en-6-one (**123**), and 17β-hydroxyandrost-4,6-dien-3-one

(**124**). For example, the presence of glucose and cycloheximide favored the obtaining of **123**, while the production of **124** was achieved in the presence of rifampicin [79]. The products isolated from the biotransformation of **12** using *Chaetomium sp.* were **93** (21%), **94** (39%), and **99** (19%); after 24 h of incubation, the presence of **11** was detected. Janeczko et al. [73] concluded that the steric factors associated with the substrate determine the location and orientation of the hydroxyl group. For example, the carbonyl group in C-17 at **11** directs the entry of the hydroxyl group at C-14 with α orientation, while the hydroxylation in C-6β is favored by the presence of the hydroxyl group in C-17, as in **12**. In the case of progesterone (**1**), which has an acyl group, dihydroxylated derivatives were observed in C-6 and C-14 (**Figure 15**) [73].

Incubation of **11** and **12** with *C. lini* ST-1 displayed different catalytic characteristics. Biotransformation of **11** afforded two products: 15α-hydroxyandrost-4-en-3,17-dione (**117**, 5%) and 11α,15α-dihydroxyandrost-4-en-3,17-dione (**125**, 64%), while **12** yielded 15α-hydroxyandrost-4-en-3,17-dione (**117**, 60%). Incubation of **1** resulted in the isolation of **14**. Wu et al. [80] concluded that the different hydroxylation sites between **11** and **12** suggested that the hydroxyl group or carbonyl group on the substrate at C-17 had influence on the location of introduced hydroxyl groups (**Figure 15**).

Dehydroepiandrosterone (3β-hydroxyandrost-5-en-17-one, **126**) endogenous prohormone secreted by the adrenal glands is a precursor of androgens and estrogens. Incubation with *M. piriformis* allowed the isolation of five compounds: 3β,17β-dihydroxyandrost-5-ene (**127**), 3β,7α-dihydroxyandrost-5-en-17-one (**128**), 3β-hydroxyandrost-5-en-7,17-dione (**129**), 3β,17β-dihydroxyandrost-5-en-7-one (**130**), and 3β,7α,17β-trihydroxyandrost-5-ene (**131**). The action of the fungus was the stereospecific hydroxylated products at C-7α (**128** and **131**) and the reduction of the carbonyl group at C-17 [51]. From the microbiological transformation of **126** using *Rhizopus stolonifer*, six poducts were isolated: **127** (20%), **128** (12%), **129** (20%), 3β,17β-dihydroxyandrost-4-ene (**132**, 12%), 17β-hydroxyandrost-4-en-3-one (**133**, 34%), and 3β,11β-dihydroxyandrost-4-en-17-one (**134**, 15%) [81]. *Fusarium oxysporum* biotransformed to **126** in a mixture of four hydroxylated derivatives (**127**–**129** and **130**), which were characterized as their acetylated derivatives; the hydroxylation was favorably in C-7 stereospecifically (α orientation) in the 3β-hydroxy-Δ^5-steroids, while *Colletotrichum musae* biotransformed to **126**–**127** by reducing the carbonyl group in C-17 (F**igure 16**) [56].

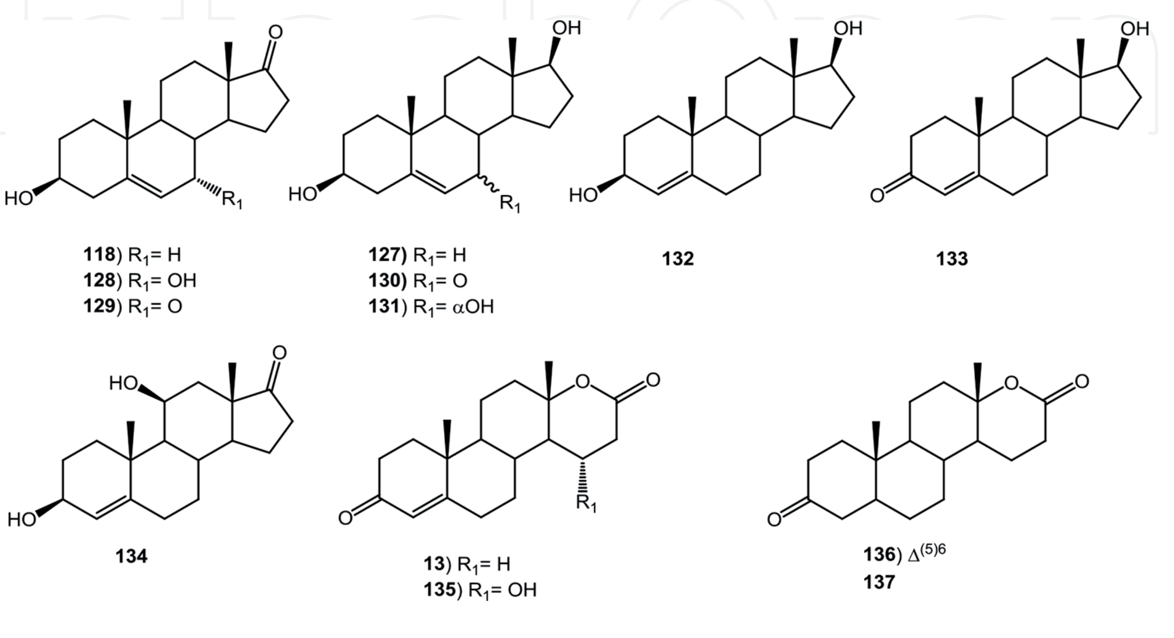


Figure 16.
*Biotransformation products of dehydroepiandrosterone (***126***).*

In the biotransformation of **126** using *Penicillium griseopurpureum* and *P. glabrum*, the following was produced; hydroxylated derivatives in C-7α (**95**), C-14α (**94**) and C-15α (**117**), with **11** being the main product. In addition, *P. griseopurpureum* generated products for the Baeyer Villiger oxidation to give the lactone D ring (testolactone, **13**) and its hydroxylated derivative at C-15α (15α-hydroxy-17α-oxa-D-homo-androst-4-en-3,17-dione, **135**); while *P. glabrum* generated the compounds, 3β-hydroxy-17α-oxa-D-homo-androst-5-en-17-one (**136**) and 3β-hydroxy-17α-oxa-D-homo-5α-androstan-17-one (**137**) (**Figure 16**) [82].

The biotransformation of 17α-ethynyl-17β-hydroxyandrost-4-en-3-one (ethisterone, **138**) and 17α-ethyl-17β-hydroxyandrost-4-en-3-one (**139**) was described using the fungi *Cephalosporium aphidicola* and *Cunninghamella elegans*. The bioconversion of **138** using *C. aphidicola* yielded 17α-ethynyl-17β-hydroxyandrost-1,4-dien-3-one (**140**, 5.5%), while by using *C. elegans*, 17α-ethynyl-11α,17β-dihydroxyandrost-4-en-3-one (**141**, 3.4%) was obtained. The biotransformation of **138** using *C. aphidicola* generated 17α-ethyl-17β-hydroxyandrost-1,4-dien-3-one (**142**, 2.2%). In contrast, when incubating **139** with *C. elegans*, two new products were obtained: 17α-ethyl-11α,17β-dihydroxyandrost-4-en-3-one (**143**, 2.8%) and 17α-ethyl-6α,17β-dihydroxy-5α-androstan-3-one (**144**, 1.6%) (**Figure 17**) [83].

Adrenosterone (**145**) is an inhibitor of the enzyme estrogen synthetase responsible for the formation of estrogen, and it has a great clinical application. Biotransformation of **145** using *C. aphidicola* produced androst-1,4-dien-3,11,17-trione (**146**, 3%), 17β-hydroxyandrost-4-en-3,11-dione (**147**, 2%), and 17β-hydroxyandrost-1,4-dien-3,11-dione (**148**, 17%). **145** (11.2%) and **12** (8.1%) were obtained from the biotransformation of **145** using *Fusarium lini*, while **147** (36.8%) was obtained from the biotransformation of **145** using *Trichothecium roseum* (**Figure 18**) [84].

The biotransformation of mesterolone (1α-methyl-17β-hydroxy-5α-androst-3-one, **149**), a synthetic androgenic steroid, was performed using different fungi as described by Choudhary et al. [85]. From the biotransformation of **149** using *C. aphidicola*, the compounds 1α-methyl-5α-androst-3,17-dione (**150**), 1α-methyl-5α-androst-3,17-diol (**151**), and 1α-methyl-15α-hydroxy-5α-androst-3,17-dione (**152**) were obtained. Incubation of **149** with *Fusarium lini* produced the compounds **152**, 1-methyl-5α-androst-1-en-3,17-dione (**153**), 1α-methyl-6α,17β-dihydroxy-5α-androst-3-one (**154**), 1α-methyl-15α,17β-dihydroxy-5α-androst-3-one (**155**), and 1-methyl-15α,17β-dihydroxy-5α-androst-1-en-3-one (**156**). The products obtained from the biotransformation of **149** using *R. stolonifer* were **150**, **154**, **156**, 1α-methyl-7α,17β-dihydroxy-5α-androst-3-one (**157**), and 1α-methyl-11α,17β-dihydroxy-5α-androst-3-one (**158**) [85]. Bioconversion of **149** using *C. blakesleeana* produced

17α-ethyl-17β-hydroxyandrost-4-en-3-one (**139**).

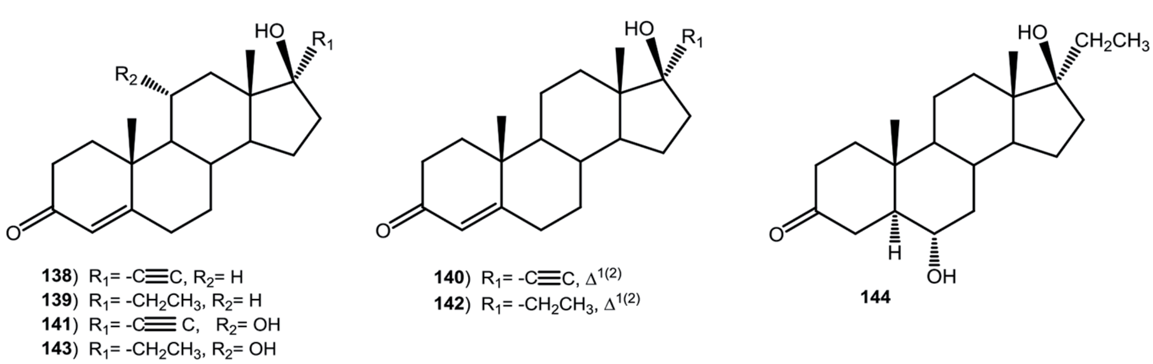
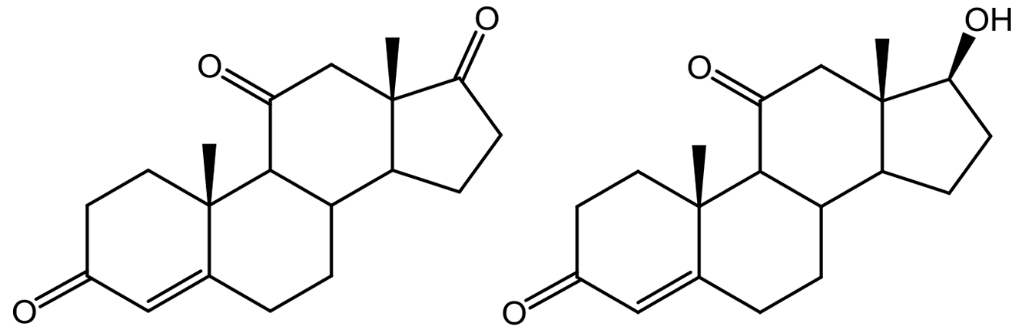


Figure 17.
*Biotransformation products of 17α-ethynyl-17β-hydroxyandrost-4-en-3-one (**138**) and 17α-ethyl-17β-hydroxyandrost-4-en-3-one (**139**).*



145
146) $\Delta^{1(2)}$

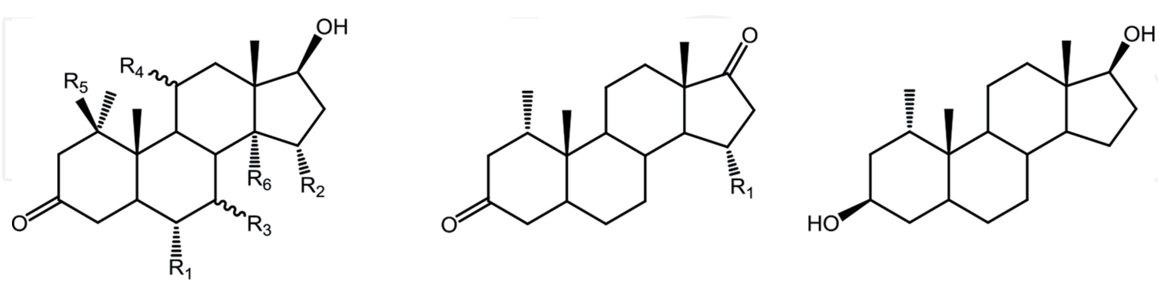
147
148) $\Delta^{1(2)}$

Figure 18.
*Biotransformation products of andresterone (**145**).*

seven biotransformation products, such as **154**, **157, 158**, in addition to 1α-methyl-1β,11β,17β-trihydroxy-5α-androst-3-one (**159**), 1α-methyl-7α,11β,17β-trihydroxy-5α-androst-3-one (**160**), 1α-methyl-1β,6α,17β-trihydroxy-5α-androst-3-one (**161**), and 1α-methyl-1β,11α,17β-trihydroxy-5α-androst-3-ona (**162**). *Macrophomina phaseolina* biotransformed **149** to obtain 1α-methyl-17β-hydroxy-5α-androst-3,6-dione (**155**) [86]. Additionally, the biotransformation of **141** using *C. blakesleeana* (ATCC 8688A) yielded three metabolites: 1α-methyl-11β,14α,17β-trihydroxy-5α-androstan-3-one (**163**, 0.4%), 1α-methyl-7β,17β-dihydroxy-5α-androstan-3-one (**164**, 0.47%), and 1α-methyl-17β-hydroxy-5α-androstan-3,7-dione (**165**, 0.67%). *C. blakesleeana* catalyzed the β-hydroxylation in C-11, and dihydroxylation and oxidations at various positions of steroid skeleton (**Figure 19**) [87].

In the microbiological transformation of 3-hydroxyestra-1,3,5-(10)-trien-17-one (**166**) using *Fusarium oxysporum* var. *cubense*, the compounds, reduced in C-17 (3,17-dihydroxyestra-1,3,5-(10)-triene, **167**) and hydroxylated in C-15 (3,15α-dihydroxiestra-1,3,5-(10)-triene, **168**), were isolated (**Figure 20**) [56].

Prednisone (**169**) is a synthetic corticosteroid (prodrug) used for the treatment of autoimmune, inflammatory and kidney diseases, among others.

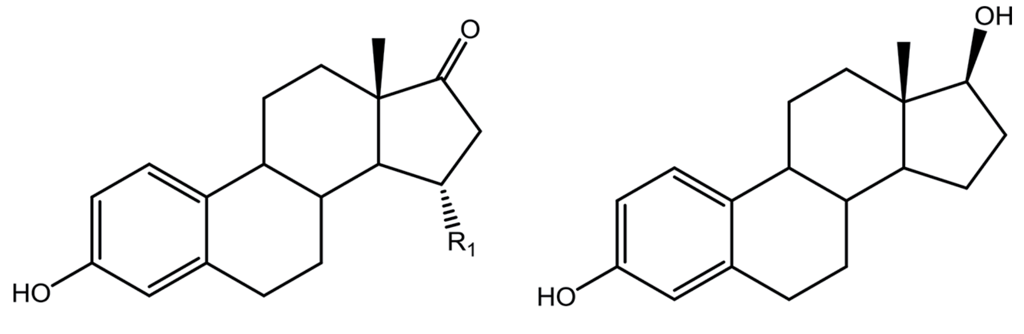


149) $R_1=R_2=R_3=R_4=R_5=R_6=$ H
154) $R_1=$ OH, $R_2=R_3=R_4=R_5=R_6=$ H
155) $R_1=R_3=R_4=R_5=R_6=$ H, $R_2=$ OH
157) $R_1=R_2=R_4=R_5=R_6=$ H, $R_3=$ OH
158) $R_1=R_2=R_3=R_5=R_6=$ H, $R_4=$ αOH
159) $R_1=R_2=R_3=R_6=$ H, $R_4=$ βOH, $R_5=$ OH
160) $R_1=R_2=R_5=R_6=$ H, $R_3=$ OH, $R_4=$ βOH
161) $R_1=R_5=$ OH, $R_2=R_3=R_4=R_6=$ H
162) $R_1=R_2=R_3=R_6=$ H, $R_4=$ αOH, $R_5=$ OH
163) $R_1=R_2=R_3=R_5=$ H, $R_4=$ αOH, $R_6=$ OH
164) $R_1=R_2=R_4=R_5=R_6=$ H, $R_3=$ βOH
165) $R_1=R_2=R_4=R_5=R_6=$ H, $R_3=$ O

150) $R_1=$ H
152) $R_1=$ OH
153) $R_1=$ H, $\Delta^{1(2)}$
156) $R_1=$ OH, $\Delta^{1(2)}$

151

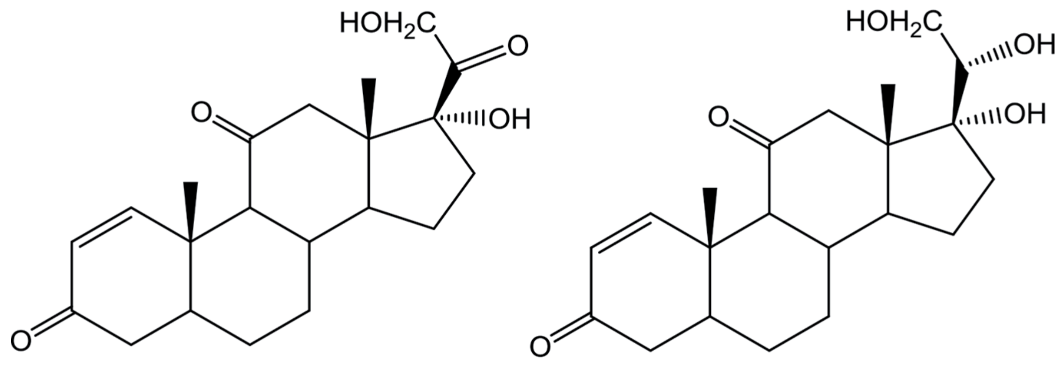
Figure 19.
*Biotransformation products of mesterelone (**149**).*



166) R_1= H
168) R_1= OH

167

Figure 20.
*Biotransformation products of 3-hydroxy-1,3,5-(10)-trien-17-one (**166**).*



169) $\Delta^{4(5)}$
170

171) R_1= H
172) $\Delta^{4(5)}$

Figure 21.
*Biotransformation products of prednisone (**169**).*

Biotransformation of **169** using *C. elegans* occurred by hydrogenation of the $\Delta^{4(5)}$ and reduction of C-20, to produce the compounds 17α,21-dihydroxy-5α-pregn-1-en-3,11,20-trione (**170**, 15.6%) and 17α,(20S),21-trihydroxy-5α-pregn-1-en-3,11-dione (**171**, 6.5%); whereas as the only biotransformation product, **169** using *F. lini* (5.2%), *R. stolonifer* (5.5%) and *C. lunata* (6.2%), was 1,4-pregnadien-17α,(20S),21-trihydroxy-3,11-dione (**172**) (**Figure 21**) [88].

The main chemical transformation carried out by different *Acremonium* species in various steroid compounds have been oxidations, reductions, hydroxylations in different positions, isomerizations, and hydrolysis of the chain in C-17. Hydrocortisone (**173**) is an important anabolic, used clinically as anti-inflammatory and antiallergic drug, besides being a raw material for the synthesis of many steroid hormones. Biotransformation of **173** using *Acremonium strictum* generated the products 11β,17β-dihydroxyandrost-4-en-3-one (**174**, 8%), 11β,17α,20β,21-tetrahydropregn-4-en-3-one (**175**, 11.2%), and 21-acetoxy-17β,17α,20-trihydroxypregn-4-en-3-one (**176**, 7.6%); it was observed that the actions of the said species were as the reduction, acetylation and degradation of the chain in C-17, without modification of the unsaturated ketone-α,β [89]. Biotransformation of **173** using *Gibberella fujikuroi* yielded 11β-hydroxyandrost-4-en-3,17-dione (**177**, 41%), while *B. subtilis* and *R. stolonifer* yielded **175** (15%). The products **173** (45%) and 3β,11β,17α,21-tetrahydroxy-5α-pregnan-20-one (**178**, 31%) were obtained from the bioconversion of **173** using *Bacillus cereus* (**Figure 22**) [90].

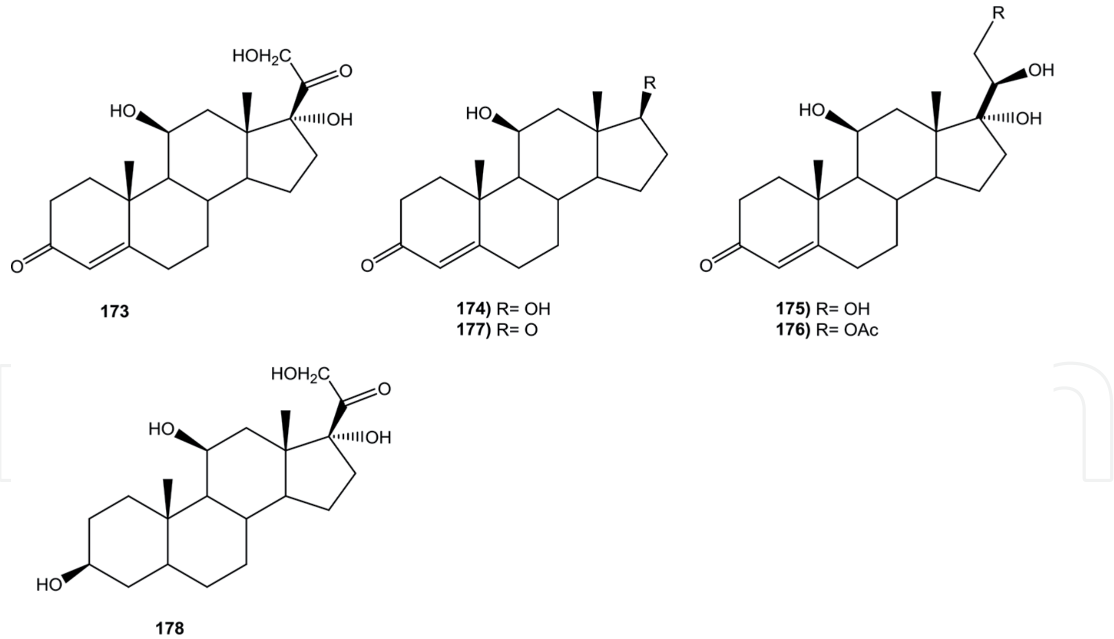


Figure 22.
*Biotransformation products of hydrocortisone (**173**).*

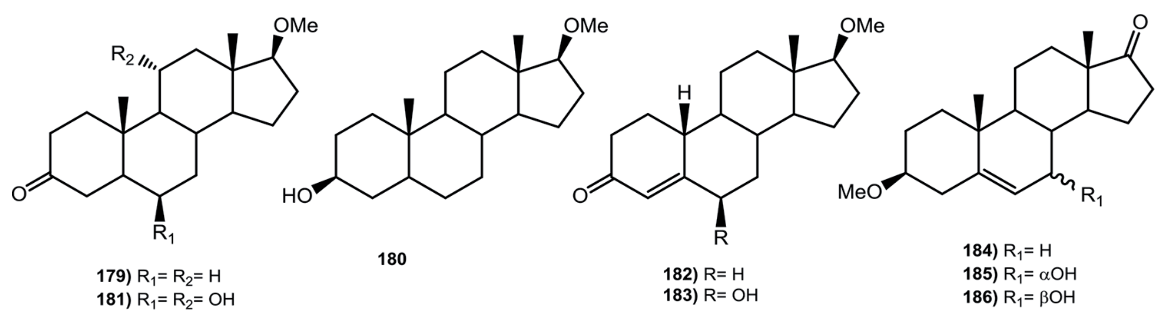


Figure 23.
*Biotransformation products of 17β-methoxy-5α-androst-3-one (**179**).*

Incubation of 17β-methoxy-5α-androst-3-one (**179**) with *Cephalosporium aphidicola* produced 17β-methoxy-5α-androst-3β-ol (**180**) and 6β,11α-dihydroxy-17β-methoxy-5α-androst-3-one (**181**); while the biotransformation of 17β-methoxyestra-4-en-3-one (**182**) using *C. aphidicola* produced a major metabolite 6β-hydroxy-17β-methoxyestra-4-en-3-one (**183**). Similarly, the microbiological transformation of 3β-methoxyandrost-5-en-17-one (**184**) gave a mixture of products: 7α-hydroxy-3β-methoxyandrost-5-en-17-one (**185**) and 7β-hydroxy-3β-methoxyandrost-5-en-17-one (**186**) (**Figure 23**) [91].

In the literature, several species of fungi belonging to the genera *Aspergillus*, *Fusarium*, *Mortierella,* and *Penicillium* and capable of hydroxylating various steroids in C-15 have been described. For example, Jekkel et al. [92] described that more than 3000 fungi hydroxylate 13β-ethyl-4-gonene-3,17-dione (**187**) in C-15 position, the genus being *Fusarium*, particularly *F. nivale*; the fungus preferentially hydroxylated **187** with an α orientation in C-15 (15α-hydroxy-13β-ethyl-4-gonene-3,17-dione, **188**, 77%) and C-7β (7β,15α-dihydroxy-13β-ethyl-4-gonene-3,17-diona, **189**). On the other hand, the biotransformation of **187** using *Mortierella pusilla* produced **188**, **190** (10β-hydroxy-13β-ethyl-4-gonene-3,17-dione) and **191** (6β-hydroxy-13β-ethyl-4-gonene-3,17-dione) (**Figure 24**).

The ethynodiol diacetate (**192**) is a synthetic derivative **1**, used as an oral contraceptive because it inhibits the ovulation process. The microbiological transformation of **192** using *Cunninghamella elegans* produced four hydroxylated compounds

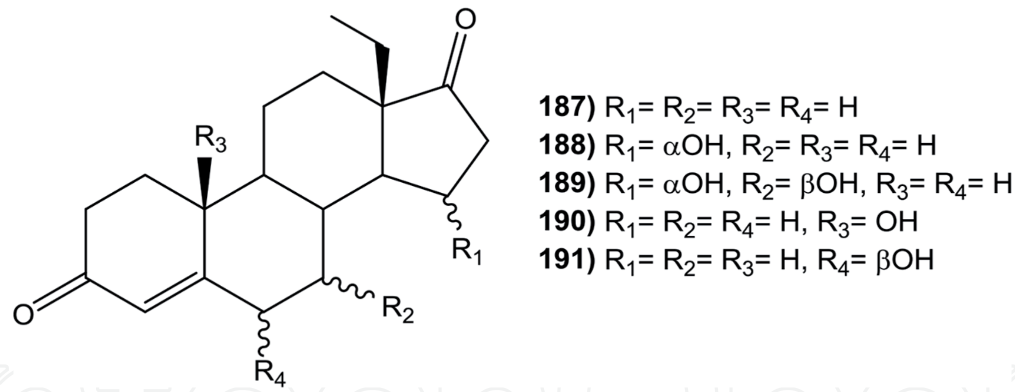


Figure 24.
Biotransformation products of 13β-ethyl-4-gonene-3, 17-dione (187).

characterized as: 17α-ethynylestr-4-en-3β,17β-diacetoxy-6α-ol (**193**, 0.5%), 17α-etynylestr-4-en-3β,17β-diacetoxy-6β-ol (**194**, 1.0%), 17α-etynylestr-4-en-3β,17β-diacetoxy-10β-ol (**195**, 0.5%), and 17α-ethynyl-17β-acetoxiestr-4-en-3-one (**196**, 1.4%) (**Figure 25**) [93].

Desogestrel (13-ethyl-17-methylene-18,19-dinor-17α-pregn-4-en-20-yn-17-ol, **197**) is an orally active third-generation contraceptive steroid drug. Conversion of **197** by *C. blackesleeana* (ATCC 8688 A) yielded four metabolites: 13-ethyl-11-methylene-18,19-dinor-17α-pregn-4-en-20-yn-6β,15β,17β-triol (**198**), 13-ethyl-11-methylene-18,19-dinor-17α-pregn-4-en-20-yn-3β,6β,17β-triol (**199**), 13-ethyl-11-methylene-18,19-dinor-17α-pregn-4-en-20-yn-3α,5α,6β,17β-tetraol (**200**), and 13-ethyl-11-methylene-18,19-dinor-17α-pregn-4-en-20-yn-6β,17β-dihydroxy-3-one (**201**). Compounds **197** and **198** showed a potent growth inhibition against drug-resistant strains of *S. aureus* (**Figure 26**) [94].

The drugs mexrenone (**202**) and canrenone (**203**) are steroids with a spironolactone in C-17 and are potent antagonists of mineralocorticoids [95]. The biotransformation of **202** and **203** using a wide variety of microorganisms resulted in the production of monohydroxylated products in different positions, where *Beauveria bassiana* generated 11α-hydroxymexrenone (**204**, 67%) as the major product, while 12β-hydroxymexrenone (**205**, 50%) and 6β-hydroxymexrenone (**206**, 33%) were obtained using *Mortierella isabellina*. The dehydrogenation product ($\Delta^{1(2)}$-mexrenone, **207**, 15%) was favored with *Bacterium* cyclooxidants as well as *Rhodococcus equi*, *Nocardia aurentia*, and *Comamonas testosteroni*. From the biotransformation of **203** using *Corynespora cassiicola*, 9α-hydroxycanrenone

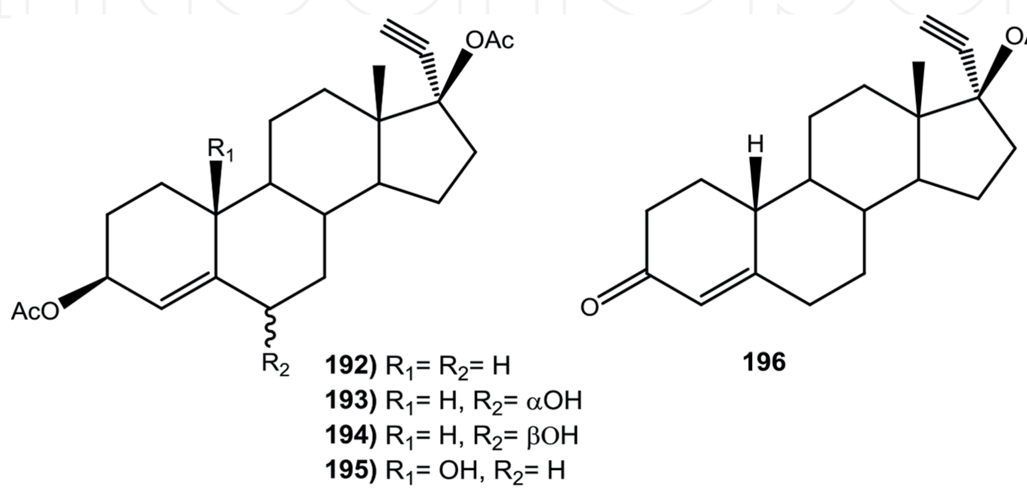
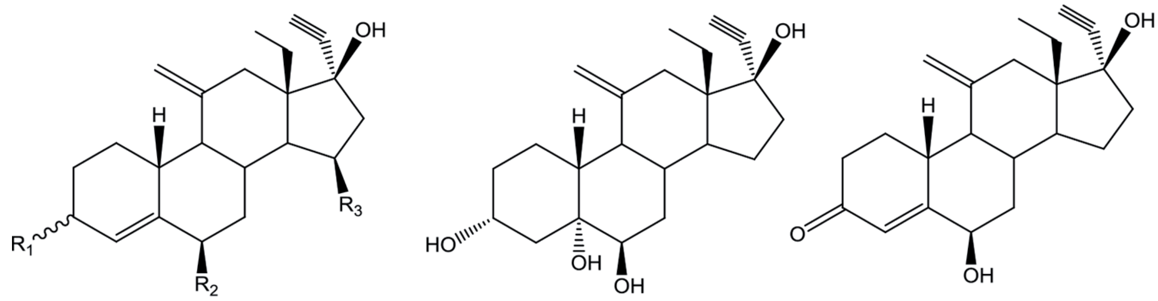


Figure 25.
Biotransformation products of ethynodiol diacetate (192).



197) R_1= R_2= R_3= H
198) R_1= H, R_2= R_3= OH
199) R_1= βOH, R_2= OH, R_3= H

200

201

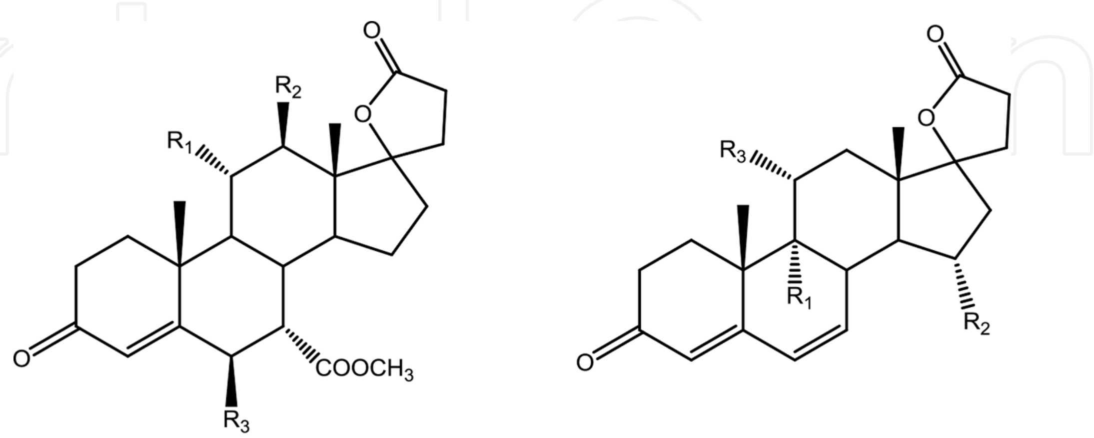
Figure 26.
*Biotransformation products of desogestrel (**197**).*

(**208**, 30%) was obtained, [96]. Conversion of canrenone (**203**) by *Colletotrichum lini* ST-1 gave two hydroxyl compounds, 15α-hydroxy-canrenone (**209**, 22%) and 11α,15α-dihydroxy-canrenone (**210**, 47%) (**Figure 27**) [80].

One of the steroids used in the treatment of breast cancer is exemestane (**211**), an inhibitor of steroidal aromatase. From the transformation of **211** using *Macrophomina phaseolina*, 16β,17β-dihydroxy-6-methylene-androsta-1,4-diene-3-one (**212**), 17β-hydroxy-6-methylene-androsta-1,4-diene-3,16-dione (**213**), and 17β-hydroxy-6-methylene-androsta-1,4-diene-3-one (**214**) were obtained, while by using *Fusarium lini*, the only product obtained was 11α-hydroxy-6-methylene-androsta-1,4-diene-3,17-dione (**215**) (**Figure 28**) [97].

4-Hydroxyandrost-4-ene-3,17-dione (formestane, **216)** is an irreversible aromatase inhibitor and therapeutically used in breast cancer treatment in post-menopausal women. Bioconversion of **216** using *Rhizopus oryzae* (ATCC 1145) resulted in the production of 4β,5α-dihydroxyandrost-3,17-dione (**217**, 8.6%) and 3,5α-dihydroxyandrost-2-ene-4,17-dione (**218**) [98], while the biotransformation of **217** using *Beauveria bassiana* produced 4,17β-dihydroxyandrost-4-en-3-one (**219**, 5.3%), 3α,17β-dihydroxy-5β-androstan-4-one (**220**, 0.9%), and 4,11α,17β-trihydroxyandrost-4-en-3-one (**221**, 2.4%) (**Figure 29**) [99].

Methyltestosterone (**222**), an anabolic steroid, was transformed by *Mucor racemosus* in 5 days to produce two monohydroxylated



202) R_1= R_2= R_3= H
204) R_1= OH, R_2= R_3= H
205) R_1= R_3= H, R_2= OH
206) R_1= R_2= H, R_3= OH
207) R_1= R_2= R_3= H, $\Delta^{1(2)}$

203) R_1= H
208) R_1= OH, R_2= R_3= H
209) R_1= R_3= H, R_2= OH
210) R_1= H, R_2= R_3= OH

Figure 27.
*Biotransformation products of mexrenone (**202**) and canrenone (**203**).*

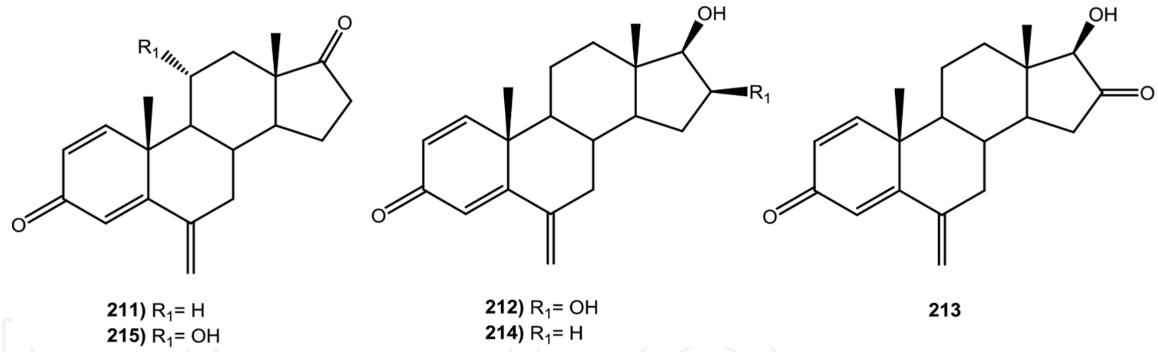


Figure 28.
Biotransformation products of exemestane (211).

products in the C-7 (7α-hydroxymethyltestosterone, **223**, 35%) and C-15 (15α-hydroxymethyltestosterone, **224**, 21%) positions, plus a dihydroxylated product (12,15α-dihydroxymethyltestosterone, **225**, 22%) [100]. Recently, three additional products were identified: 11α-hydroxy-17α-methyltestosterone (**226**), 6β-hydroxy-17α-methyltestosterone (**227**), and 6β,11α-dihydroxy-17α-methyltestosterone (**228**). Isolation of hydroxylation products have been reported in different carbons from **222** with different orientations, C-6β, C-7β, C-9α, C-11α, C-12β, and C-15α (**Figure 30**).

Dianabol (methandrostenolone, 17α-methyl-17β-hydroxyl-androst-1,4-dien-3-on, **229**) is an oral anabolic steroid that promotes the synthesis of proteins (increasing the muscle tissue). From the biotransformation of **229** using *Cunninghamella elegans*, five bioconversion products were obtained: 6β-hydroxydianabol (**230**), 15α-hydroxydianabol (**231**), 11α-hydroxydianabol (**232**), 6β,12β-dihydroxydianabol (**233**), and 6β,15α-dihydroxydianabol (**234**). The products 17β-hydroxy-17α-methyl-5α-androst-1,4-dien-3,6-dione (**235**), 7β-hydroxydianabol (**236**), 15β-hydroxydianabol (**237**), 17β-hydroxy-17α-methyl-5α-androst-1,4-dien-3,11-dione

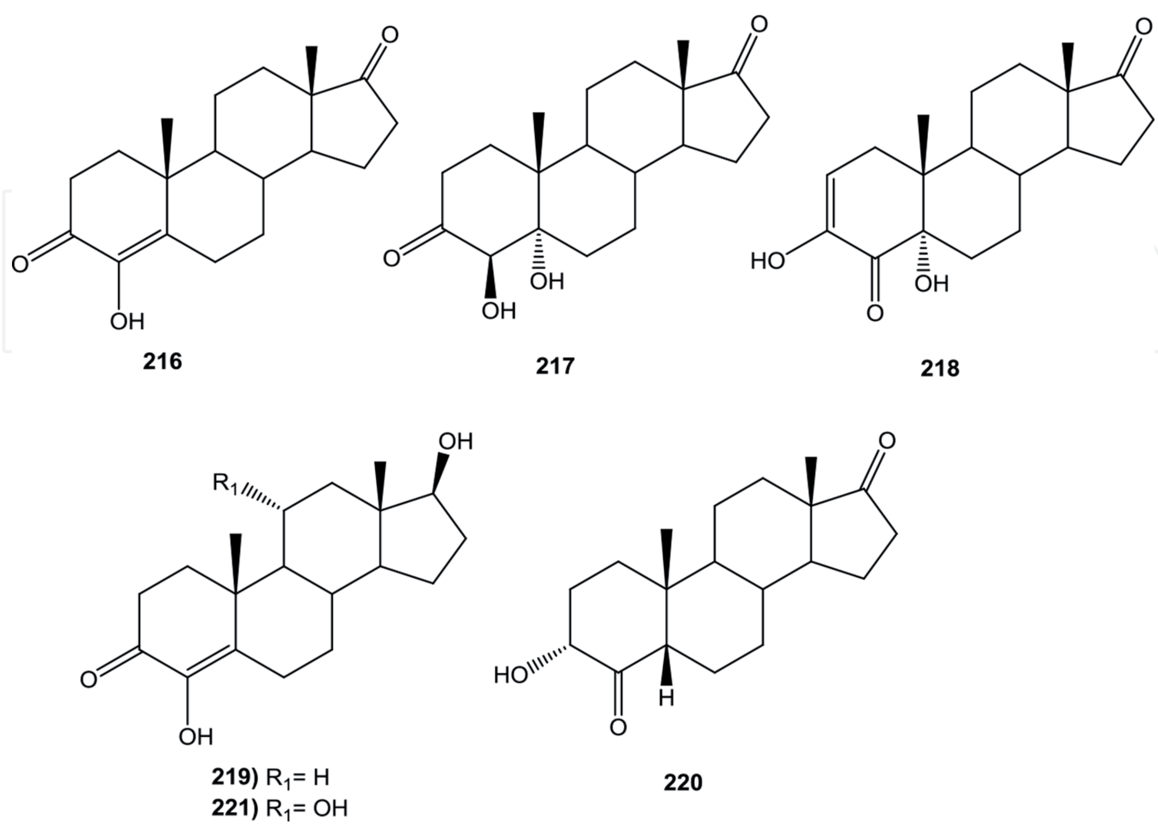


Figure 29.
Biotransformation products of formestane (216).

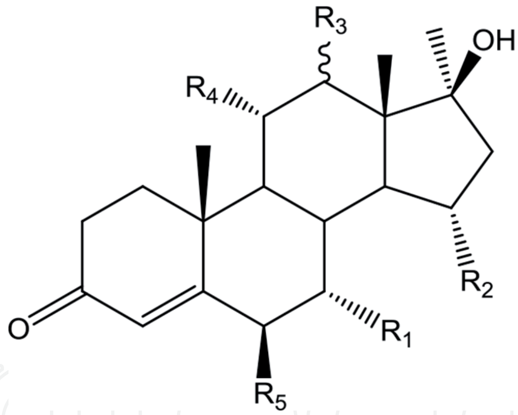


Figure 30.
*Biotransformation products of methyltestosterone (**222**).*

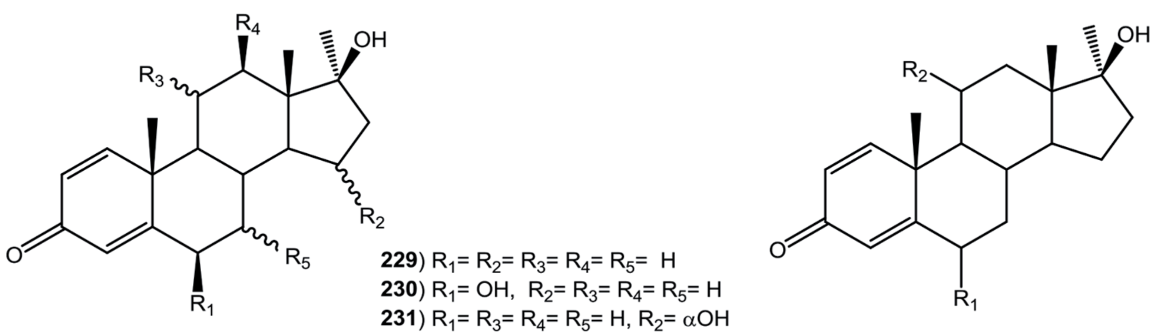
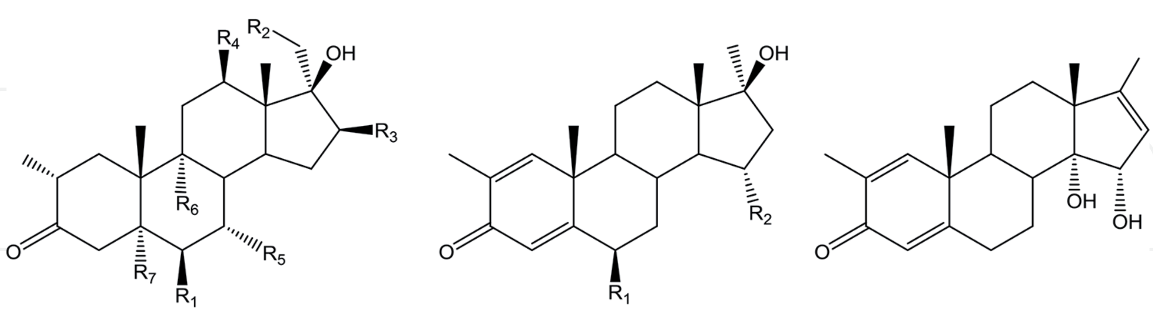


Figure 31.
*Biotransformation products of dianabol (**229**).*



251

Figure 32.
*Biotransformation products of masthasterone (**241**).*

(**238**), and 11β-hydroxydianabol (**239**) were obtained from the biotransformation of **229** using *Macrophomina phaseolina* [101]. Biotransformation of **229** using several microorganisms has been reported, for example, *Penicillium notatum* [102] transformed **229** into **230** and **231**, while *Trichoderma hamatum* produced **232** [103].

Similarly, *B. bassiana, A. ochraceus*, *Colletotrichum lagenarium,* and *Sporotrichum sulfurreducens* gave a biotransformed product **232** [104]. *Absidia glauca* metabolized **229** in compounds **230**, and **236–237** [105]. In contrast, the biotransformation of **229** using *A. coerula* yielded **239** along with 7α-hydroxydianabol (**240**) [106], while by using *B cinerea*, **237** was obtained as the only product (**Figure 31**) [107].

Methasterone (**241**) is a synthetic anabolic steroid, known to gain muscle mass. Microbial transformation of **241** using *M phaseolina* yielded 17β-hydroxy-17α(hydroxymethyl)-2α-methyl-5α-androstane-3,6-dione (**242**), while by using *C. blakesleeana*, 7α-hydroxymethasterone (**243**, 2.0%), 7α,16β-dihydroxymethasterone (**244**, 0.7%), 5α,12β-dihydroxymethasterone (**245**, 1.0%), 7α,12β-dihydroxymethasterone (**246**, 1.5%), and 7α,9α-dihydroxy-methasterone (**247**, 0.5%) were obtained. Incubation of **241** with *Fusarium lini* yielded different metabolites with dehydrogenation in ring A and D: 6β,17β-dihydroxy-2,17α-dimethyl-5α-androst-1,4-diene-3-one (**248**, 1.0%), 15α,17β-dihydroxy-2α,17α-dimethyl-5α-androst-1,4-diene-3-one (**249**, 0.6%), 6β,17β-dihydroxy-2,17α-dimethylandrost-1,4-diene-3-one (**250**, 0.4%), 14α,15α-dihydroxy-2,17-dimethyl-5α-androst-1,4,16-trien-3-one (**251**, 0.3%), 17β-hydroxy-2,17α-dimethyl-5α-androst-5α-1,4-dien-3,6-dione (**252**, 0.3%), and 17β-hydroxy-2,17α-dimethyl-5α-androst-1,4-dien-3-one (**253**, 1.0%) (**Figure 32**) [108].

3. Conclusions

The biotransformation processes of different steroid compounds described in this review, although not exhaustive, aim to highlight the importance of biotransformation through different microorganisms, as a useful chemical-biological tool for obtaining novel derivatives for research purpose and as industrial applications. An example includes obtaining steroid compounds for the pharmaceutical industry.

Biotransformation of steroids has been implemented in an important way in the partial synthesis of new steroids, for their evaluation as hormones and drugs. Currently, there is a wide variety of steroids used as diuretics, anabolic, anti-inflammatory, antiandrogenic, anticontraceptive, antitumor, among other applications. Chemical functionalization in different carbon atoms of the sternum skeleton is related to the biological activity of the molecule. This is why microbiological transformations play an important role in obtaining these compounds through chemical transformations, such as the oxidation of hydroxyl group at C-3 and C-17, isomerization of the double bond $\Delta^{5(6)}$ to $\Delta^{4(5)}$, hydrogenation of double bonds $\Delta^{1(2)}$ and $\Delta^{4(5)}$, and reduction of the carbonyl group at C-17 and C-20 with β orientation. Biohydroxylations performed in different positions of the steroid skeleton—C-11α, C-11β, C-15β, and C-16α—using different species of fungi of the genera *Rhizopus*, *Aspergillus*, *Curvularia*, *Cunninghamella*, and *Streptomyces* with high yields are an important chemical transformation in many synthesis schemes of new steroids with a determined biological activity.

Hydroxylation of steroids—progesterone, testosterone, 17α-methyltestosterone, and 4-androsten-3,17-dione—presenting the 4-en-3-one system, proceeds with a high stereo- and regioselectivity in the C-6 and C-11 positions, with a β orientation in C-6 and α orientation in C-11. The presence of the methyl group in C-10 is necessary for the hydroxylation in C-11, as can be seen in the derivatives of 19-nortesterone.

The interest in the biotransformation of steroid compounds has been increasing in recent years, due to the obtaining of new and useful pharmacologically active compounds. In addition to the development of new genetically modified strains, there is an increase in the availability of immobilized enzymes and the manipulation of culture media.

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Biotransformation of steroids proceeds with low to moderate yields in general. One of the main causes is their low solubility in water. Currently, methodologies are developed that allow the incorporation of chemicals—surfactants, ionic liquids, cyclodextrins, liposomes, among others—that contribute to improve the yields of each biotransformation process and the processes friendly to the environment.

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