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Microbial Biotransformation of Some Anabolic Steroids

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ABSTRACT

Microbial biotransformations of various anabolic steroids are reviewed. Studies on oxidation, reduction, and carbon bond cleavage are highlighted. Various anabolic steroid substrates, their metabolites and the microorganisms used for the biotransformations are compiled covering the literature from the period 1984−2018.
Keywords: Microbial biotransformation; Review; Anabolic steroidal substrate; Metabolite; Compilation.

1. Introduction

Microorganisms have been used extensively for the hydroxylation of steroids since their enzymes catalyze reactions with high regio- and stereospecifity. Their ability to oxidize steroidal compounds has immense synthetic and commercial importance. This was realized for the first time in 1952 when Murray and Peterson of Upjohn Company patented the process of 11α-hydroxylation of progesterone by a *Rhizopus* species [1]. Since then, steroids subjected to microbial biotransformations have proliferated in order to obtain new steroidal derivatives for evaluation as drugs and hormones.

The importance of anabolic steroids lies in their therapeutic use in medicine to stimulate muscle growth in patients with AIDS [2] and treat severe burn injury, trauma and chronic infections [3]. There are many reviews on microbial biotransformation of steroids [4−8]. However, no reviews on the microbial biotransformation of anabolic steroids have been recently reported in the literature.

The areas which are now receiving attention in microbial biotechnology are: application of newer concepts of genetic engineering of microorganisms with improved characteristics such as the production of artificial insulin by the genetic modification of *Escherichia coli* [9], solubility enhancement for carrying out biotransformation of substrates that are insoluble in water by using different media, including aqueous, aqueous: organic and organic solvents, gas: solid systems, supercritical fluids and ionic liquids [10], immobilization of enzymes or whole cells in a suitable matrix for economic utilization [10], development of a continuous process for better and economic product recovery such as the microbial production of vanillin which has been successfully used in the food industry [11]; and manipulation of culture media for improvement in product yields by use of cyclodextrin [7].

In this review, our interest lies in the preparation of novel steroids that are difficult to synthesize by chemical means. Microbial transformations of two-twenty anabolic steroids (androstenediol (**1**), androstenedione (**2**), 4-chloro-17α-methyl testosterone (**3**), 4-chlorotestosterone (**4**), 4-

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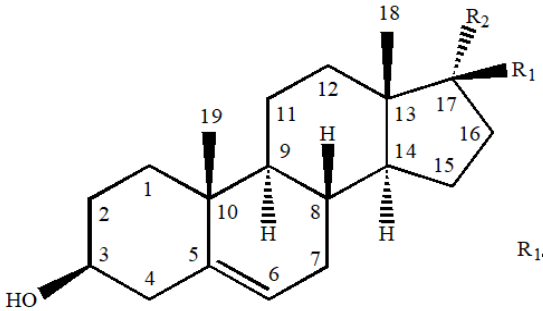
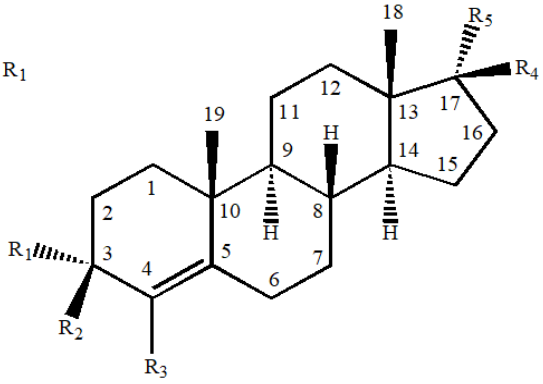
chlorotestosterone acetate (**5**), dehydroepiandrosterone (**6**), 1-dehydro-17α-methyltestosterone (**7**), 1-dehydrotestosterone (**8**), ethylestrenol (**9**), 17α-ethyl-19-nortestosterone (**10**), mestanolone (**11**), mesterolone (**12**), methandienone (**13**), 4-methoxytestosterone (**14**), methyltestosterone (**15**), 4-methyltestosterone (**16**), 17-methyl-1-testosterone (**17**), nandrolone (**18**), nor androstenedione (**19**), oxandrolone (**20**), oxymetholone (**21**) and testosterone (**22**)) (Figures–1 and –2) are reviewed here.





Androstenediol (**1**): R_1 = OH, R_2 = H

Androstenedione (**2**): $R_1 = R_2 = R_4 = R_5$ = C=O, R_3 = H

Dehydroepiandrosterone (**6**): $R_1 = R_2$ = C=O

4-Chloro-17α-methyltestosterone (**3**): $R_1 = R_2$ = C=O, R_3 = Cl, R_4 = OH, $R_5 = CH_3$

4-Chlorotestosterone (**4**): $R_1 = R_2$ = C=O, R_3 = Cl, R_4 = OH, R_5 = H

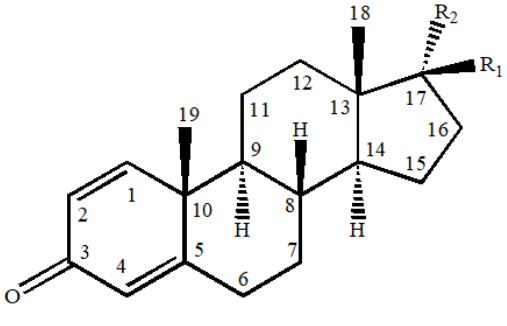
4-Chlorotestosterone acetate (**5**): $R_1 = R_2$ = C=O, R_3 = Cl, $R_4 = OCOCH_3$, R_5 = H

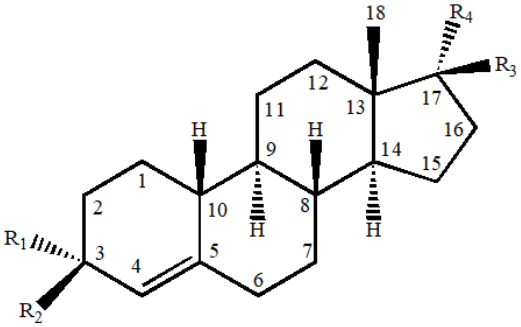
4-Methoxytestosterone (**14**): $R_1 = R_2$ = C=O, $R_3 = OCH_3$, R_4 = OH, R_5 = H

Methyltestosterone (**15**): $R_1 = R_2$ = C=O, R_3 =H, R_4 = OH, $R_5 = CH_3$

4-Methyltestosterone (**16**): $R_1 = R_2$ = C=O, $R_3 = CH_3$, R_4 = OH, R_5 = H

Testosterone (**22**): $R_1 = R_2$ = C=O, $R_3 = R_5$ = H, R_4 = OH





1-Dehydro-17α-methyltestosterone (**7**): R_1 = OH, R_2 = CH_3

1-Dehydrotestosterone (**8**): R_1 = OH, R_2 = H

Methandienone (**13**): R_1 = OH, R_2 = H

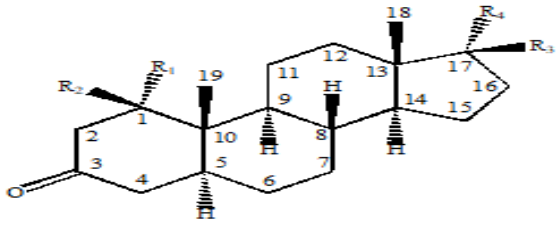
Ethylestrenol (**9**): R_1 = R_2 = H, R_3 = OH, R_4 = C_2H_5

17α-Ethyl-19-nortestosterone (**10**): R_1 = R_2= C=O, R_3 = OH, R_4 = C_2H_5

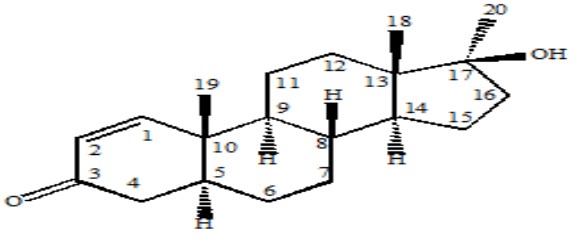
Nandrolone (**18**): R_1 = R_2 = C=O, R_3 = OH, R_4 = H

Norandrostenedione (**19**): R_1 = R_2 = R_3 = R_4 = C=O

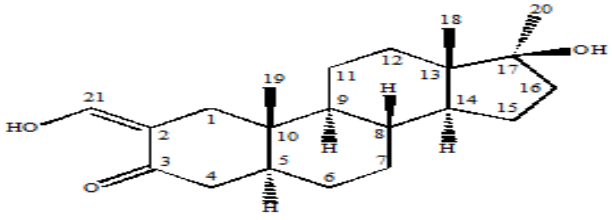
Fig. 1: Anabolic steroid substrates used in this review.



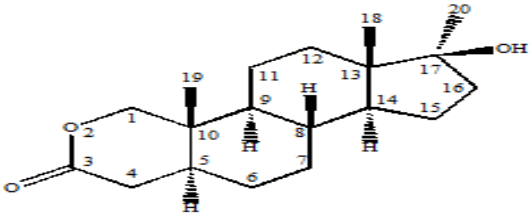
Mestanolone (**11**): R_1 = R_2 = H, R_3 = OH, R_4 = CH_3
Mesterolone (**12**): R_1 = CH_3, R_2 = H, R_3 = OH, R_4 = H



17-Methyl-1-testosterone (**17**)



Oxymetholone (**21**)



Oxandrolone (**20**)

Fig. 2: Anabolic steroid substrates used in this review.

Studies on oxidation, reduction and carbon–carbon bond cleavage are compiled; including the microorganism used, the product obtained and the reference as well (Tables 1–3).

This review attempts to present the situation during the period from 1984 to 2018.

2. Results and Discussion

Large scale experiments showed that microbial oxidations of various anabolic steroids **1**–**22** by different microorganisms were predominant, including regio-selective hydroxylations at C–6, C–7, C–11, C–12, C–14, C–15 positions on steroidal skeletons with high stereospecifity, dehydrogenations between carbons 1–2, 4–5, regiospecific keto formations at C–3, C–7 C–11, C–17, and Baeyer-Villiger lactonizations at C–17 (Table-1). On the other hand, reductions of some anabolic steroids such as **2**, **4**, **6**, **8**, **12**, **19**, **21** and **22** were also obtained in smaller numbers of metabolites as compared to oxidations, including reductions of ketones to alcohols and hydrogenations of olefinic carbons between carbons 4–5 and 6–7 (Table–2). Carbon-carbon bond cleavages, especially decarboxylations at C–17, have been performed on dianabol (**13**) and 17-methyl-1-testosterone (**17**) by *Rhizopus stolonifer*, and Oxymetholone (**21**) by *Fusarium lini* (Table–3). Structures of metabolite**s** were deduced through comparative spectroscopic studies with substrates **1**–**22**.

2.1. Oxidation

Most studies of microbial oxidations on anabolic steroids describe the hydroxylation process. The 6α-, 7α-, 11α- and 15α-hydroxylations are now extensively achieved by microbial transformations with high yields and minimum costs. For instance, the biotransformation of mestanolone (**11**) by *Rhizopus stolonifer* yielded two metabolites with 11.4% and 18.0% yields [25]. Other hydroxylations that seem to have an attention in industries are 9α-, 7β-, 11β-, 15β- and 16β-hydroxylations. On the other hand, the rest of oxidation studies involved Baeyer-Villiger lactonizations, keto formations, and dehydrogenations. Kolek, et al. reported one step Baeyer-Villiger lactonization of androstenediol (**1**) by *Penicillium camemberti* to yield a single metabolite (testolactone) [12]. Similarly, Al-Aboudi, et al. produced testolactone from testosterone (**22**) by the plant pathogen fungus, *Rhizopus stolonifer* [34], while **22** was subjected to dehydrogenation easily by *Fusarium lini* to form 1-dehydrotestosterone with a high regiosepecifity [34]. Oxidation studies on microbial transformations of substrates **1-22** that included microorganisms, metabolites and references are compiled (Table 1). In this review, the most useful microorganisms subjected to the oxidation of anabolic steroids are fungi. Fungi are an extremely diverse group of organisms. Among them, plant pathogen fungi are causing diseases associated with roots such as wilts and rots. The plant pathogen fungus *Rhizopus stolonifer* had the largest contribution in the oxidation of anabolic steroids followed by *Fusarium culmorum* and the entomopathogenic fungus *Beauveria bassiana*, respectively.

Table 1. Oxidation

Substrate	Microorganism	Product	% Yield *	Reference
Androstenediol (**1**)	*Penicillium camemberti*	Testolactone		[12]
	Mortierella isabellina	(i) 3β,7α,17β-Trihydroxyandrost-5-ene (ii) 3β,7β,17β-Trihydroxyandrost-5-ene (iii) 3β,17β-Dihydroxyandrost-5-en-7-one		[13]
Androstenedione (**2**)	*Paecilomyces victoriae*	(i) 7α-Hydroxyandrostenedione (ii) 7α-Hydroxy-17α-methyl testosterone		[14]
	Phycomyces blakesleeanus	14α-Hydroxytestosterone		[15]
4-Chloro-17α-methyltestosterone (**3**)	*Fusarium culmorum*	(i) 6β-Hydroxy-4-chloro-17α-methyltestosterone (ii) 15α-Hydroxy-4-chloro-17α-methyltestosterone		[16]
4-Chlorotestosterone (**4**)	*Fusarium culmorum*	(i) 6β-Hydroxy-4-chloroandrostenedione (ii) 15α-Hydroxy-4-chloroandrostenedione (iii) 3β,15α -Dihydroxy-4-chloro- 4-androstene-17-one (iv) 3β,15α-Dihydroxy-4α-chloro-5α-androstan-17-one		[16]
4-Chlorotestosterone acetate (**5**)	*Fusarium culmorum*	3β,15*α*-Dihydroxy-4*α*-chloro-5*α*-androstan-17-one		[17]
Dehydroepiandrosterone (DHEA) (**6**)	*Rhizopus stolonifer*	(i) 17β-Hydroxyandrost-4-ene-3-one (ii) 3β,11β-Dihydroxyandrost-4-ene-17-one (iii) 3β,7α-Dihydroxyandrost-5-ene-17-one (iv) 3β,7α,17β-Trihydroxyandrost-5-ene (v) 11β-Hydroxyandrost-4,6-diene-3,17-dione		[18, 19]

Substrate	Microorganism	Product	% Yield *	Reference
	Macrophomina phaseolina	(i) Androstane-3,17-dione (ii) Androst-4-ene-3,17-dione (iii) Androst-4-ene-17β-ol-3-one (iv) Androst-4,6-diene-17β-ol-3-one (v) Androst-4-ene-3β-ol-6,17-dione (vi) Androst-4-ene-3β,7β,17β-triol (vii) Androst-5-ene-3β,7α,17β-triol		[20]
	Mucor piriformis	(i) 3β-Hydroxyandrost-5-ene-7,17-dione (ii) 3β,17β-Dihydroxyandrost-5-en-7-one (iii) 3β,7α-Dihydroxyandrost-5-en-17-one (iv) 3β,7α,17β-Trihydroxyandrost-5-ene		[21]
	Penicillium griseopurpureum Smith	(i) Androst-4-ene-3,17-dione (ii) 17a-Oxa-D-homo-androst-4-ene-3,17-dione (testololactone) (iii) 15α-Hydroxyandrost-4-en-3,17-dione (iv) 15α-Hydroxy-17a-oxa-D-homo-androst -4-ene-3,17-dione (v) 14α-Hydroxyandrost-4-en-3,17-dione (vi) 7α-Hydroxyandrost-4-en-3,17-dione		[22]
	Penicillium glabrum (Wehmer)	(i) Androst-4-ene-3,17-dione (ii) 17a-Oxa-D-homo-androst-4-ene-3,17-dione (testololactone) (iii) 3β-Hydroxy-17a-oxa-D-homo-androst-5-en-17-one (iv) 3β-Hydroxy-17a-oxa-D-homo-5α-androstan-17-one		[22]
	Beauveria bassiana	(i) 5-Androsten-3β,11α,17β-triol (ii) 7α-Hydroxy dehydroepiandrosterone		[23]
1-Dehydro-17α-methyltestosterone (**7**)	*Beauveria bassiana*	11α-Hydroxy-1-dehydro-17α-methyltestosterone		[23]

Substrate	Microorganism	Product	% Yield *	Reference
1-Dehydrotestosterone (**8**)	*Beauveria bassiana*	(i) 11α-Hydroxy-1-dehydrotestosterone (ii) 11α-Hydroxyandrost-1,4-diene-3,17-dione (iii) 11α-Hydroxytestosterone (iv) 11α-Hydroxyandrost-4-ene-3,17-dione		[23]
Ethylestrenol (**9**)	*Rhizopus stolonifer*	(i) 17α-Ethyl-3β,17β-dihydroxy-19-norandrost-4-ene (ii) 17α-Ethyl-17β-hydroxy-19-norandrost-4-en-3-one		[24]
17α-Ethyl-19-nortestosterone (**10**)	*Fusarium culmorum*	(i) 6β-Hydroxy-17α-ethyl-19-nortestosterone (ii) 15α-Hydroxy-17α-ethyl-19-nortestosterone (iii) 11α-Hydroxy-17α-ethyl-19-Nortestosterone		[16]
Mestanolone (**11**)	*Rhizopus stolonifer*	(i) 11α-Hydroxymestanolone (11α,17β-dihydroxy-17α-methyl-5α-androstan-3-one) (ii) 6α-Hydroxymestanolone (6α,17β-dihydroxy-17α-methyl-5α-androstan-3-one)	11.4 %	[25]
	Macrophomina phaseolina	(i) 17β-Hydroxy-17α-methyl-5α-andros-1-en-3,11-dione (ii) 14α,17β-Dihydroxy-17α-methyl-5α-androstan-3,11-dione (iii) 17β-Hydroxy-17α-methyl-5α-andros-1,14-dien-3,11- dione (iv) 17β-Hydroxy-17α-methyl-5α-androstan-3,11- dione (v) 11α-Hydroxymestanolone (11α,17β-dihydroxy-17α-methyl-5α-androstan-3-one)	0.9 % 1.6 % 0.3 % 0.78 % 5.6 %	[26]

Substrate	Microorganism	Product	% Yield *	Reference
	Cunninghamella blakesleeana	(i) 9α,11β-Dihydroxymestanolone (9α,11β,17β-trihydroxy-17α-methyl-5α-androstan-3-one) (ii) 2β,11α-Dihydroxymestanolone (2β,11α,17β-trihydroxy-17α-methyl-5α-androstan-3-one)	0.7 % 0.92 %	[26]
Mesterolone (**12**)	*Cunninghamella blakesleeana*	(i) 1α-Methyl-1β,11β,17β-trihydroxy-5α-androstan-3-one (ii) 1α-Methyl-7α,11β,17β-trihydroxy-5α-androstan-3-one (iii) 1α-Methyl-1β,6α,17β-trihydroxy-5α-androstan-3-one (iv) 1α-Methyl-1β,11α,17β-trihydroxy-5α-androstan-3-one (v) 1α-methyl-11α,17β-dihydroxy-5α-androstan-3-one (vi) 1α-methyl-6α,17β-dihydroxy-5α-androstan-3-one (vii) 1α-methyl-7α,17βdihydroxy-5α-androstan-3-one		[26]
	Macrophomina phaseolina	1α-Methyl,17β-hydroxy-5α-androstan-3,6-dione		[26]
	Cephalosporium aphidicola	(i) (1α, 5α)-1-Methylandrostane-3,17-dione (ii) (1α, 5α, 15α)-15-Hydroxy-1-methylandrostane-3,17-dione		[27]
	Fusarium lini	(i) (5α)-1-Methylandrost-1-en-3,17-dione (ii) (1α, 5α, 6α, 17β)-6,17-Dihydroxy-1-methylandrostan-3-one (iii) (1α, 5α, 15α, 17β)-15,17-Dihydroxy-1-methylandrostan-3-one (iv) (5α, 15α, 17β)-15,17-Dihydroxy-1-methylandrost-1-en-3-one		[27]

Substrate	Microorganism	Product	% Yield *	Reference
	Rhizopus stolonifer	(i) (1α, 5α)-1-Methylandrostane-3,17-dione (ii) (5α)-1-Methylandrost-1-en-3,17-dione (iii) (1α, 5α, 6α, 17β)-6,17-Dihydroxy-1-methylandrostan-3-one (iv) (1α, 5α, 7α, 17β)-7,17-Dihydroxy-1-methylandrostan-3-one (v) (1α, 5α, 11α, 17β)-11,17-Dihydroxy-1-methylandrostan-3-one (vi) (5α, 15α, 17β)-15,17-Dihydroxy-1-methylandrost-1-en-3-one		[27]
Methandienone (methandrostenolone, dianabol) (**13**)	*Rhizopus stolonifer*	11α,17β-Dihydroxy-androsta-1,4-diene-3-one		[25]
	Cunninghamella elegans	(i) 6β,17β-Dihydroxy-17α-methylandrost-1,4-dien-3-one (ii) 15α,17β-Dihydroxy-17α-methylandrost-1,4-dien-3-one (iii) 11α,17β-Dihydroxy-17α-methylandrost-1,4-dien-3-one (iv) 6β,12β,17β-Trihydroxy-17α-methylandrost-1,4-dien-3-one (v) 6β,15α,17β-Trihydroxy-17α-methylandrost-1,4-dien-3-one		[28]
	Macrophomina phaseolina	(i) 17β-Hydroxy-17α-methylandrost-1,4-dien-3,6-dione (ii) 7β,17β-Dihydroxy-17α-methylandrost-1,4-dien-3-one (iii) 15β,17β-Dihydroxy-17α-methylandrost-1,4-dien-3-one (iv) 17β-Hydroxy-17α-methylandrost-1,4-dien-3,11-dione (v) 11β,17β-Dihydroxy-17α-methylandrost-1,4-dien-3-one		[28]
4-Methoxytestosterone (**14**)	*Fusarium culmorum*	6β-Hydroxy-4-methoxyandrostenedione		[16]

Substrate	Microorganism	Product	% Yield *	Reference
Methyltestosterone (**15**)	*Mucor racemosus*	(i) 7α-Hydroxymethyltestosterone (ii)15α-Hydroxymethyl testosterone (iii)12α,15α-Dihydroxymethyl testosterone	35.0 % 21.0 % 22.0 %	[29]
	Fusarium culmorum	(i) 6β-Hydroxy-17α-methyltestosterone (ii) 15α-Hydroxy-17α-methyltestosterone (iii) 12β-Hydroxy-17α-methyltestosterone		[16]
	Beauveria bassiana	11*α*-Hydroxy-17*α*-methyl testosterone		[23]
4-Methyltestosterone (**16**)	*Fusarium culmorum*	(i) 6β-Hydroxy-4-methylandrostenedione (ii) 6β-Hydroxy-4-methyltestosterone		[16]
17-Methyl-1-testosterone (**17**)	*Rhizopus stolonifer*	(i) Methandrostenolone (17β-hydroxy-17α-methylandrost-1,4-diene-3-one) (ii) 11α,17β-Dihydroxy-androsta-1,4-diene-3-one	 18.0 %	[25]
Nandrolone (19-Nortestosterone) (**18**)	*Rhizopus stolonifer*	(i) 19-Norndrost-4-en-3,17-dione (ii) 6α,17β-Dihydroxy-19-norndrost-1,4-dien-3-one		[24]
	Beauveria bassiana	11α -Hydroxy-19-nortestosterone		[23]
	Cunninghamella echinulata	(i) 10β,12β,17β-trihydroxy-19-nor-4-androsten-3-one (ii) 10β,16α,17β-trihydroxy-19-nor-4-androsten-3-one (iii) 6β,10β,17β-trihydroxy-19-nor-4-androsten-3-one (iv) 10β,17β-dihydroxy-19-nor-4-androsten-3-one (v) 6β,17β-dihydroxy-19-nor-4-androsten-3-one		[30]
	Cunninghamella blakesleeana	(i) 6β,10β,17β-trihydroxy-19-nor-4-androsten-3-one (ii) 10β,17β-dihydroxy-19-nor-4-androsten-3-one (iii) 10β-hydroxy-19-nor-4-androsten-3,17-dione (iv) 16β,17β-dihydroxy-19-nor-4-androsten-3-one		[30]

Substrate	Microorganism	Product	% Yield *	Reference
Norandrostenedione (**19**)	*Fusarium culmorum*	(i) 6β-Hydroxy-19-nortestosterone (ii) 6β-Hydroxy-19-norandrostenedione		[16]
	Corynespora melonis	9α-Hydroxy-19-norandrostenedione		[31]
	Nocardia restrictus	9α-Hydroxy-19-norandrostenedione		[31]
Oxandrolone (**20**)	*Rhizopus stolonifer*	(i) 11α-Hydroxyoxandrolone (11α,17β-Dihydroxy-17α-methyl-2-oxa-5α-androstan-3-one) (ii) 6α-Hydroxyoxandrolone (6α,17β-Dihydroxy-17α-methyl-2-oxa-5α-androstan-3-one) (iii) 9α-Hydroxyoxandrolone (9α,17β-Dihydroxy-17α-methyl-2-oxa-5α-androstan-3-one)	25.0 % 5.0 % 8.0 %	[32]
Oxymetholone (**21**)	*Macrophomina phaseolina*	(i) 17β-Hydroxy-2-(hydroxymethyl)-17α-methyl-5α-androstan-1-en-3-one (ii) 2α,17α-Di(hydroxymethyl)-5α-androstan-3β-17β-diol		[33]
	Rhizopus stolonifer	2α,17α-Di(hydroxymethyl)-5α-androstan-3β-17β-diol		[33]
	Fusarium lini	(i) 17β-Hydroxy-2-(hydroxymethyl)-17α-methyl-5α-androstan-1-en-3-one (ii) 17α-Methyl-5α-androstan-2α,3β-17β-triol (iii) 17β-Hydroxy-2-(hydroxymethyl)-17α-methylandrost-1,4-dien-3-one		[33]
Testosterone (**22**)	*Beauveria bassiana*	(i) 11α-Hydroxytestosterone (ii) 5α-Androstan-11α,17β-diol-3-one (iii) 11α-Hydroxyandrost-4-ene-3,17-dione (iv) 5α-Androstan-11α-ol-3,17-dione		[23]

Substrate	Microorganism	Product	% Yield *	Reference
	Rhizopus stolonifer	(i) Androst-4-en-3,17-dione (ii) Testolactone (iii) 17β-Hydroxy-5α-androstan-1,6-dione (iv) 11α-Hydroxyandrost-4-en-3,17-dione (v) 11α-Hydroxytestolactone		[34]
	Fusarium lini	(i) Androst-4-en-3,17-dione (ii) Androst-1,4-dien-3,17-dione (iii) 1-Dehydrotestosterone (17β-Hydroxyandrost-1,4-dien-3-one) (iv) 11α-Hydroxyandrost-1,4-dien-3,17-dione (v) 11α-Hydroxytestosterone (11α,17β-Dihydroxyandrost-4-en-3-one) (vi) 11α,17β-Dihydroxyandrost-1,4-dien-3-one		[34]
	Curvularia lunata	17-Dehydrotestosterone (androst-4-ene-3,17-dione)		[35]
	Pleurotus oestreatus	15α-Hydroxytestosterone (15α,17β-dihydroxyandrost-4-en-3-one)		[35]
	Aspergillus famigatus	15β-Hydroxytestosterone		[36]
	Phycomyces blakesleeanus	(i) 6β-Hydroxytestosterone (ii) 7α-Hydroxytestosterone (iii) 1-Dehydroandrostenedione (androsta-l,4-diene-3,17-dione) (iv) 1-Dehydrotestosterone (17β-hydroxyandrosta-1,4-diene-3-one) (v) Androstenedione		[15]

* Available % yields in the literature

2.2. Reduction

The reduction of anabolic steroids by microorganisms has been also reviewed. Reductions of anabolic steroids by microorganisms involved transformations of ketones to alcohols and hydrogenations. Ahmad et al. reported the reduction of 3-keto to 3α-hydroxy form in mesterolone (**12**) by *Cephalosporium aphidicola* to produce a single metabolite with a high stereospecifity [27], while Choudhary, et al. reported the hydrogenation between C-5 and C-6 on dehydroepiandrosterone (**6**) by *Macrophomina phaseolina* to form androstanedione (**2**) [20]. Studies on microbial reductions of some anabolic steroids that included microorganisms, metabolites and references are compiled (Table-2).

Table 2. Reduction

Substrate	Microorganism	Product	% Yield *	Reference
Androstenedione (**2**)	*Phycomyces blakesleeanus*	(i) Testosterone (ii) 14α-Hydroxytestosterone	19.0 %	[15]
4-Chlorotestosterone (**4**)	*Fusarium culmorum*	(i) 3β,15α -Dihydroxy-4-chloro- 4-androstene-17-one (ii) 3β,15α-Dihydroxy-4α-chloro-5α-androstan-17-one		[16]
Dehydroepiandrosterone (DHEA) (**6**)	*Rhizopus stolonifer*	(i) 3β,17β-Dihydroxyandrost-5-ene (ii) 3β,17β-Dihydroxyandrost-4-ene		[18]
	Macrophomina phaseolina	(i) Androstane-3,17-dione (ii) Androst-4-ene-17β-ol-3-one (iii) Androst-4,6-diene-17β-ol-3-one (iv) Androst-5-ene-3β,17β−diol (v) Androst-4-ene-3β,7β,17β-triol (vii) Androst-5-ene-3β,7α,17β-triol		[20]
	Mucor piriformis	(i) 3β,17β-Dihydroxyandrost-5-ene (ii) 3β,17β-Dihydroxyandrost-5-en-7-one (iii) 3β,7α,17β-Trihydroxyandrost-5-ene		[21]
	Penicillium glabrum (Wehmer)	3β-Hydroxy-17a-oxa- D-homo-5α-androstan-17-one		[22]
	Beauveria bassiana	Androstenediol		[23]
1-Dehydrotestosterone (**8**)	*Beauveria bassiana*	(i) 11α-Hydroxytestosterone (ii) 11α-Hydroxyandrost-4-ene-3,17-dione		[23]
Mesterolone (**12**)	*Cephalosporium aphidicola*	(1α, 3β, 5α, 17β)-1-Methylandrostane-3,17-diol		[27]
Norandrostenedione (**19**)	*Fusarium culmorum*	6β-Hydroxy-19-nortestosterone		[16]

Substrate	Microorganism	Product	% Yield *	Reference
Oxymetholone (**21**)	*Macrophomina phaseolina*	(i) 17β-Hydroxy-2α-(hydroxymethyl)-17α-methyl-5α-androstan-3-one (ii) 2α-(Hydroxymethyl)-17α-methyl-5α-androstan-3β-17β-diol		[33]
	Aspergillus niger	(i) 17β-Hydroxy-2α-(hydroxymethyl)-17α-methyl-5α-androstan-3-one (ii) 2α-(Hydroxymethyl)-17α-methyl-5α-androstan-3β-17β-diol		[33]
	Rhizopus stolonifer	(i) 2α,17α-Di(hydroxymethyl)-5α-androstan-3β-17β-diol (ii) 17β-Hydroxy-2α-(hydroxymethyl)-17α-methyl-5α-androstan-3-one		[33]
	Fusarium lini	(i) 17β-Hydroxy-2-(hydroxymethyl)-17α-methyl-5α-androstan-1-en-3-one (ii) 17α-Methyl-5α-androstan-2α,3β-17β-triol (iii) 17β-Hydroxy-2-(hydroxymethyl)-17α-methylandrost-1,4-dien-3-one		[33]
Testosterone (**22**)	*Beauveria bassiana*	(i) 5α-Androstan-11α,17β-diol-3-one (ii) 5α-Androstan-11α-ol-3,17-dione		[23]

* Available % yields in the literature

2.3. Carbon-carbon bond cleavage

The carbon-carbon bond cleavage in anabolic steroids that took place by microorganisms has been compiled. The process includes full oxidations of methyl carbons to carboxylic acids. Carboxylic acids are easily eliminated in the form of CO_2 (g). For instance, Mohammad, et al. reported the demethylation at C-17 on 17-methyl-1-testosterone (**17**) by *Rhizopus stolonifer* to form androstenedione (**2**) with 18.0% yield [25]. However, decarboxylation was performed at C-2 rather than C-17 on oxymetholone (**21**) by *Fusarium lini* to yield 17α-Methyl-5α-androstan-2α,3β-17β-triol [33]. Studies on carbon-carbon bond cleavage of anabolic steroids by microorganisms are compiled (Table-3).

Table 3. Carbon-carbon bond cleavage

Substrate	Microorganism	Product	% Yield *	Reference
Methandienone (Methandrostenolone, dianabol) (**13**)	*Rhizopus stolonifer*	11α,17β-Dihydroxy-androsta-1,4-diene-3-one		[25]
17-Methyl-1-testosterone (**17**)	*Rhizopus stolonifer*	11α,17β-Dihydroxy-androsta-1,4-diene-3-one	18.0 %	[25]
Oxymetholone (**21**)	*Fusarium lini*	17α-Methyl-5α-androstan-2α,3β-17β-triol		[33]

* Available % yields in the literature

3. General Experimental Methods

3.1. Applications of microorganisms

3.1.1. Microorganisms and culture medium

Microorganisms are grown on potato dextrose-agar or sabouraud glucose agar at 25 °C, and stored at 4 °C. The media for microorganism differ from one organism to another, but generally the following ingredients are used in distilled H_2O: glucose, peptone, yeast extract, KH_2PO4, glycerol, KCl, $MgSO_4.7H_2O$, and NaCl [34].

3.1.2. Fermentation and extraction conditions

The medium is distributed into conical flasks and then sterilized in an autoclave at 121° C for 15 minutes. Mycelia are inoculated into all the flask media, and the flasks are placed in an incubator with rotary shaking at 28° C. After the complete growth of microorganism, substrate is dissolved in a particular organic solvent that is not toxic to microorganism, and then equally distributed to each cultural flask and flasks are again placed on incubated shaker to allow the occurrence of fermentation. An additional flask labeled as a negative control, which contained a microorganism without substrate, is placed with the incubated flasks under the same conditions, and another additional flask, labeled as a positive control, which contained a substrate added to the medium without microorganisms, is also placed with the incubated flasks. After the completion of fermentation, the mycelia are separated from the medium by filtration and then the medium is placed in a separatory funnel for extraction. The metabolites are extracted using a suitable organic solvent. This extraction is repeated three times. The crude extract containing the metabolites is collected by evaporating the organic solvent, using vacuum on rotavap, and then analyzed by TLC [34].

3.1.3. Isolation of transformed products

Different chromatographic techniques can be used to isolate the metabolites [30-39]. The crude extract is adsorbed on silica and subjected to column chromatography. The metabolites are eluted and purified by solvent mixtures of different polarities.

3.1.4. Structural elucidations of metabolites

Structures of the metabolites are elucidated through comparative spectroscopic studies (UV, FT-IR, 1D-NMR, 2D-NMR, MS) with the substrate [30-37].

3.2. Applications of immobilized enzymes onto support materials

3.2.1. Support materials

The support (carrier) can be a synthetic organic polymer such as acrylic resins [40], a biopolymer such as cellulose, starch, agarose, carragenans, and chitosan [41], or an inorganic solid such as alumina, silica, zeolites, and mesoporous silicas [42]. A variety of matrixes have been used as support materials for enzyme immobilization [43].

3.2.2. Immobilization of enzymes onto solid supports

Enzyme immobilization onto solid supports is a possible alternative to in-solution digestion. Different reactive groups of the supporting material (–OH, $–NH_2$, and –COOH) can be utilized for covalent protein binding using relatively simple coupling strategies [44]. These

approaches include co-polymerization with polyacrylamide gels, binding onto microbeads, silica-based substrates, synthetic polymers, and the inner walls of open capillaries or Micro-channels in microfluidics.

4. Conclusion:

Microbial biotransformation technology has proven to be a useful tool for stereo- and regio-specific oxidations, regio-selective reductions, and carbon-carbon bond cleavages. This review attempts to present the situation during the period from 1984 to 2018, and to help researchers for choosing the suitable microorganism for stereo- and regio-selectivity reactions on other anabolic steroids.

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التحويل الحيوي الميكروبي لبعض مركبات الستيرويدات البنائية

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ملخص

في هذه الدراسة تتم مراجعة التحولات الحيوية الميكروبية لمختلف مركبات الستيرويدات البنائية. تم تسليط الضوء على دراسات حول الأكسدة، والاختزال، وانقسام رابطة الكربون والكربون. تم تجميع دراسات وتغطيتها على مختلف الستيرويدات ومستقلباتها، والكائنات الدقيقة المستخدمة في التحولات الحيوية وذلك في الفترة ما بين 1984–2018.

الكلمات الدالة: التحويل الحيوي الميكروبي، مراجعة، الستيرويد البنائي الأولي، المستقلب، تجميع.

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