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STEROID TRANSFORMATIONS BY MICROORGANISMS—III

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Key Word Index—Steroids; microbial transformation; cell immobilization; production of steroid drugs; methods for better yields of steroid metabolites.

Abstract—Microbial transformations of various steroids are reviewed with special reference to the newer technniques which are being employed for better yields of desired metabolites. The present position of microbial biotechnology in the steroid drug industry and the future possibilities are discussed. A compilation of different steroid substrates, their metabolites and the microorganisms used for the transformations is included covering the literature during the period 1984–mid 1987.

INTRODUCTION

Since the successful application of microbiology in the production of corticosteroid hormones by 11α-hydroxylation of progesterone, steroids have been the traditional field for industrially used microbial transformations. It has been reported [1] that at present the production of steroid drugs and hormones of the values of \$ 400 million requires biotechnological procedures. An excellent article by Smith [2] on biotechnology applications dealing with steroids has appeared. Biotechnology applications dealing with steroids may be broadly classified into two categories: (i) processing raw materials into useful intermediates for general steroid production, e.g. microbial hydrolysis of plant saponins to sapogenins and microbial degradation of the side chain of sterols to useful steroid intermediates; (ii) specific steroid intermediates to desired products. 11α-, 11β- and 16α-Hydroxylations, Δ^1-dehydrogenation and side chain degradations are now exclusively achieved in the steroid industry by microbial biotransformation. Total synthesis has now been used for the production of 19-norsteroid progestational agents. However, microbial fermentation technology is utilised for the resolution of the racemic intermediate mixtures to obtain the enantiomer of required configuration. There is a flurry of activity in the field of biotransformation of steroids to bring about new developments in relation to the lowering of costs in the maufacture of the products and to introduce new steroids into commerce. Less commercially oriented topics, e.g. fundamental studies of ketosteroid reductase, steroid hydroxylases, and double-bond isomerases are also receiving attention. Biotransformation of Δ^4-3-ketosteroids by the fungus *Rhizopus arrhizus* has been discussed by Holland [3] in relation to the mechanism for C-6 hydroxylation and epoxidation. The data obtained by the author's group clearly support a stepwise rather than concerted mechanism for both the hydroxlation and epoxidation reactions. An example of an enzymic oxidation is given in which the product stereochemistry is controlled by the stereoelectronic properties of the substrate, rather than by any steric control imposed upon the substrate by the enzyme. In the previous reviews [4, 5] the literature which developed during the period 1979–mid 1984 was covered ncluding citation of earlier monographs and reviews. This review attempts to present the situation reached during the period 1984–mid 1987.

Although introduction of new steroids into commerce is now limited there are continuing reports on production of new steroids by microbial transformation as this technique can conveniently be exploited for preparation of steroid analogues which are otherwise inaccessible. These are continuing efforts for the development of immobilized systems to carry out steroid transformations. However, as the industrially used microbial transformations at present are all based on enzymes with low stability, and often multiple cofactor demand, the technique of immobilization is limited to immobilization of intact cells [6]. As most of the steroid substrates are water insoluble, innovative approaches to substrate addition, e.g. as finely divided suspensions, suspensions in surface active agents, soluble complexes or derivatives of substrates and their gradual addition to the culture broth over several intervals during the fermentation period have been reported [7]. A report has described some reactions in aqueous saturated organic solvents with immobilized cells [8]. The frequent application of microbial transformation is for the introduction of a particular hydroxyl group and the introduction of the Δ^1-double bond. The synthetic protocol of a steroid drug is so designed that the microbial trsnsformation is conducted at the terminal or near-terminal step.

Hydroxylation

The introduction of a hydroxyl group at the desired position of a steroid molecule is preferably done by microbial biotechnology as every site in the molecule is accessible for microbial hydroxylation whereas chemical solutions to the problem are far from economical, if not impossible. During the period under review a large number of steroid substrates have been investigated for hydroxylation. However, the commercially important 11- and 16α-hydroxylations have received greater attention. The substrates and their metabolites are listed in Table 1.

Side chain degradation

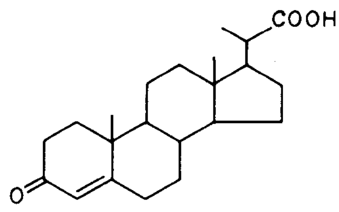
Although Snider *et al.* [9] reported a chemical method of remote oxidation for the efficient removal of the cholesterol side chain the microbial removal of the aliphatic side chain of phytosterols offers a promising method, processes for the commercial conversion of sterols to 17-ketosteroids have been developed and are also in practical use [1]. For selective cleavage of the side chain, methods for the inhibition of the ring degradation were developed. The various methods used for this inhibition have been disussed by Martin [10]. Selective cleavage of the acetyl side chain of pregnanes by microorganisms to the C-19 steroids is also commercially important as 16-dehydropregnenolone acetate (16-DPA) or progesterone are easily derived chemically from widely available steroid sapogenins. While degradation of the phytosterol side chain proceeds via β-oxidation, the cleavage of the acetyl side chain of progesterone has beenshown to be via oxygen insertion reactions of the Baeyer–Villiger type [11].

An efficient method for degradation of the side chain of sterols having branched chains at C-24 by inclusion of an exogenous source of HCO_3^- in the nutriet medium has been patented [12]. The process involved the preparation of an inoculant of *Mycobacterium* NRRL B-3805 which was used to inoculate a soybean–glucose medium. After incubation of the medium at 25° for two 48 hr stages, sitosterol suspended in Tween 80 was added with $NaHCO_3$ and incubation continued as before for 48 hr. At this time the formation of androst-4-ene-3,17-dione had reached a level of 99.0 mmol with a concentration of 25 mM HCO_3^-.

3-Oxo-4-pregnene-20-carboxylic acid (**1**) is a useful intermediate for the manufacture of steroid hormones. Microbial production of this compound from cholesterol using a strain of *Corynebacterium* has been achieved [13]. A 24 hr preculture *Corynebacterium* 320-6-2-2 was incubated in 100 ml of pH 7.2 medium containing Na_2HPO_4 (0.1 g, KH_2PO_4 (0.05 g), NH_4Cl (0.2 g), $MgSO_4{\cdot}7H_2O$ (0.05 g), NaCl (0.2 g), $FeCl_3{\cdot}6H_2O$ (0.001 g), yeast extract (0.02 g) and corn steep liquor (0.7 %) in the presence of *n*-hexadecane at 27° for 74 hr. Cholesterol (50 mg) was then added and incubation continued at 27° for 2 days. The broth was found to contain 23.3 mg of **1** per litre and small amounts of 3-oxo-1,4-pregnadiene-20-carboxylic acid (**2**) and androsta-1,4-diene-3,17-dione (ADD). The compositions of the media for the culture of mycobacteria which can split off the side chain of sitosterol have been reported by Russian workers [14]. The rate of transformation of sitosterol to ADD and compound **2** was dependent on the composition of the culture medium used. The optimum medium for *M. mucosum* 3-AP was made of 2 % wort, 1 % glucose, mineral salts and vitamins and for *M. flavum* B_2 the optimum medium contained 0.5 % glucose, sucrose, maltose or lactose as carbon source and amino N as a nitrogen source. An efficient method for biodegradation of the side chain of sterols and their mixtures to the intermediate products of steroid drug synthesis has been reported by Protiva *et al.* [15]. *Mycobacterium* CCM 3528 converted cholesterol, sitosterol, stigmasterol, the sterol fraction of a deodorizing condensate, and the sterol fracion from tall oils from paper pulp manufacture to 22-hydroxy-23,24-bisnorchola-1,4-dien-3-one (**3**) at 71, 39, 59, 48 and 64 % yields, respectively. On the other hand, *Mycobacterium* CCM 3529 converted these sterols to ADD at 65, 35, 36, 54 and 57 % yields, respectively. The sterol fraction of the deodorizing condensate produced from wastes of the fat industry contained sitosterol, campesterol, brassicasterol, stigmasterol as well as contaminated cholesterol and the sterol fraction of the tall oils was a mixture of sitosterol and campesterol. The results demonstrated that the sterol fractions obtained as industrial byproducts may conveniently be utilized for microbial production of useful steroid drug intermediates. The preparation of 12α-substituted pregna-1,4-diene-3,20-diones by a combination of microbial and chemical methods has been patented [16]. *Alcaligenes faecalis* D 4020-K 15 transformed deoxycholic acid (**4**) into 12α-hydroxy pregna-1,4-dien-3-one-20-carbaldehyde (**5**). The fermentation of compound **4** with the strain was conducted in a medium (100 ml, pH 8.4) containing compound (**4**) (1 g), glucose (0.1 g), NH_4NO_3 (0.2 g), KH_2PO_4 (0.12 g), K_2HPO_4 (0.61 g), $MgSO_4{\cdot}7H_2O$ (0.02 g), yeast extract (0.02 g) and NaOH (0.1 g) at 30° for 2 days with shaking. The intermediate (**5**) thus obtained was chemically converted to 12α-acetoxypregna-1,4-diene-3,20-dione (**6**). The microbial poduction of 11β-hydroxypregna-4-en-3-one-20-carbaldehyde (**8**) has also been patented [17]. Thus, *Alcaligenes faecalis* FERM BP-204 was incubated in a pH 8.0 medium (100 ml) containing 11β-hydroxylithocholic acid (**7**) (0.5 g), glycerol (0.5 g), NH_4NO_3 (0.1 g), KH_2PO_4 (0.1 g), K_2HPO_4 (0.6 g), magnesium citrate (0.02 g), yeast extract 0.02 g) and NaOH (0.1 g) and incubated at 30° for 2 days with shaking. The metabolite (**8**) was then isolated in the usual way.

An efficient conversion of cholesterol to 17-hydroxysteroids by the use of an external proton donor has been reported [18]. Incubation of cholesterol with *Mycobacterium* NRRL B-3805 in the presence of one equivalent to hydroquinone gave a 43 % yield of testosterone and in the presence of one equivalent of ascorbic acid gave a 40.6 % yield of testosterone.

Microbial side chain and ring fissions of phytosterols and pregnanes easily derivable from phytosterols and steroid sapogenins are also receiving attention for the preparation of useful precursors for convergent synthesis of pharmaceutically interesting retrosteroids [19]. A number of microbial strains mainly of the genera *Nocardia*, *Mycobacterium*, *Arthrobacter* and *Pseudomonas* have been found suitable for the purpose [3, 4]. A mutant strain of *Rhodococcus australis* CSIR 236.457 which accumulates 3aα-H-4α-(3′-propionic acid)-5α-hydroxy-7aβ-methylhexahydro-indan-1-one-δ-lactone (**9**) from cholesterol, stigmasterol and sitosterol was investigated by Ferreira *et al.* [20]. The indanone derivative (**9**) was produced in 60 % molar yield in a dilute black strap molasses medium containing 6-12 g cholesterol/l after 72 hr fermentation period. Side chain and ring cleavage of pregnanes by *Arthrobacter simplex* IICB-321 producing metabolites among others the indanone derivative (**9**) and 3-hydroxy-9,10-seco-1,3,5(10)-androstatriene-9,17-dione (**10**) has recently been reported [21] and pathways of formation of the metabolites have also been discussed.



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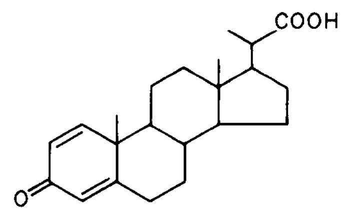

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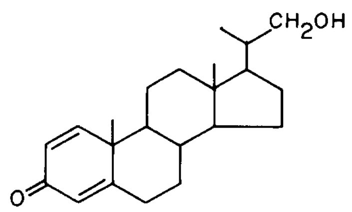

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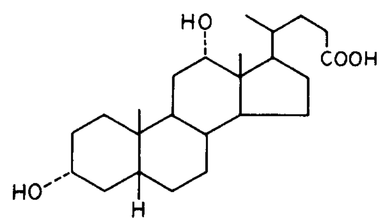

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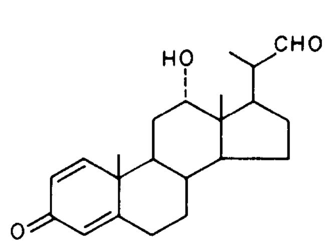

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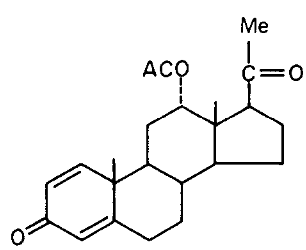

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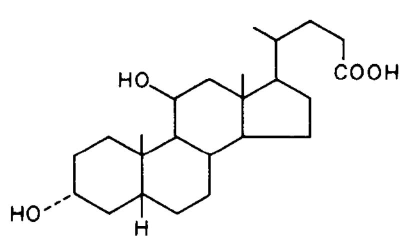

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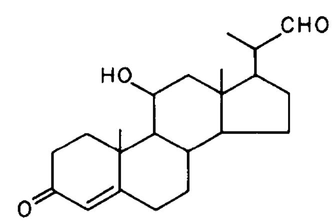

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Immobilization of microbial cells

As already mentioned the industrially used microbial steroid transformations are based on enzymes with low stability and consequently the immobilization technique is employed for immobilization of intact cells. The technology for immobilization of living cells and its application in microbial transformations of steroid hormones has been reviewed [22]. Microbial cells can be immobilized by covalent attachment to or adsorption on solid supports, entrapment in polymeric gels, or by encapsulation and cross-linking.

Sequential conversion of cortexolone (**11**) to prednisolone (**12**) by immobilized mycelia of *Curvularia lunata* and immobilized cells of *Arthrobacter simplex* has been reported [23]. Immobilized living mycelia of *C. lunata* having a high 11β-hydroxylation activity were prepared by *in situ* germination of spores entrapped in photocrosslinked resin gels of a suitable network structure. Acetone-dried cells of *A. simplex* having an induced steroid Δ^1-dehydrogenase activity were also entrapped with photo-cross-linkable resin polymers and used for Δ^1-dehydrogenation of hydrocortisone to prednisolone. The production of prednisolone from cortexolone was conveniently achieved by the combination of sequential steps, 11β-hydroxylation and subsequent Δ^1-dehydrogenation. Each immobilized microbial cell system was stable and could be used repeatedly for the sequential reactions. The opera-

Table 1. Hydroxylation

Substrate	Microorganism	Product	Reference
1β-Hydroxylation			
5α-Androstane-3,17-dione	*Cunninghamella elegans*	1β,9α-Dihydroxy-5α-androstane-3,17-dione	[42]
17β-Hydroxy-5α-androstan-3-one	*Cunninghamella elegans*	1β,14α,17β-Trihydroxy-5α-androstan-3-one	[42]
3β-Acetoxy-D-homo-5α-androstan-17a-one	*Cunninghamella elegans*	i) 1β,3β-Dihydroxy-D-homo-5α-androstane-7,17a-dione	[43]
		ii) 1β,3β,7β-Trihydroxy-D-homo-5α-homo-5α-androstane-17a-one	
		iii) 1β,3β,7α-Trihydroxy-D-homo-5α-androstan-17a-one	
3β-Hydroxy-D-homo-5α-androstan-17a-one	*Cunninghamella elegans*	i) 1β,3β-Dihydroxy-D-homo-5α-androstane-7,17a-dione	[43]
		ii) 1β,3β,7β-Trihydroxy-D-homo-5α-androstan-17a-one	
		iii) 1β,3β,7α-Trihydroxy-D-homo-5α-androstan-17a-one	
D-Homo-5α-androstane-3,17a-dione	*Cunninghamella elegans*	i) 1β,3β-Dihydroxy-D-homo-5α-androstane-7,17a-dione	
		ii) 1β,7α-Dihydroxy-D-homo-5α-androstane-3,17a-dione	[43]
		iii) 1β,7β-Dihydroxy-D-homo-5α-androstane-3,17a-dione	
2-Hydroxylation			
3,17β-Dihydroxyestra-1,3,5-triene	*Aspergillus alliaceus* UI 315	2,3,17β-Tri-hydroxyestra-1,3,5(10)-triene	[44]
2β-Hydroxylation			
17β-Acetoxy-B-homo-androsta-1,4-dien-3-one	*Rhizopus arrhizus* ATCC-11145	i) 17β-Acetoxy-2β,6β,7aβ-trihydroxyandrost-4-en-3-one	[45]
		ii) 17β-Acetoxy-2β,7aβ-dihydroxyandrost-4-en-3-one	
4-Hydroxylation			
3,17β-Dihydroxyestra-1,3,5-triene	*Aspergillus alliaceus* UI 315	3,4,17β-Trihydroxyestra-1,3,5(10)-triene	[44]
5α-Hydroxylation			
17β-Hydroxy-5α-androstan-3-one	*Cunninghamella elegans*	5α,17β-Dihydroxyandrostan-3-one	[42]
6α-Hydroxylation			
3(*N*-pyrrolideno)-Androsta-3,5-dien-17-one	*Rhizopus arrhizus* ATCC-11145	6α,Hydroxyandrost-4-ene-3,17-dione	[46]
17β-Hydroxy-5α-androstan-one	*Cunninghamella elegans*	6α,17β-Dihydroxy-5α-androstan-3-one	[42]
6β-Hydroxylation			
17β-Hydroxyandrost-4-en-3-one	*Rhizopus arrhizus* ATCC-11145	6β,17β,Dihydroxyandrost-4-en-3-one	[3]
17β-Acetoxyandrost-4-en-3-one	*Rhizopus arrhizus* ATCC-11145	17β-Acetoxy-6β-hydroxyandrost-4-en-3-one	[46]
3-(*N*-pyrrolideno)-Androsta-3,5-dien-17-one	*Rhizopus arrhizus* ATCC-11145	6β-Hydroxyandrost-4-ene-3,17-dione	[46]
Pregn-4-ene-3,20-dione	*Aspergillus ochraceus*	6β,11α-Dihydroxypregn-4-ene-3,20-dione	[47]
Pregna-4,8(9)-diene-3,20-dione	*Mucor griseocyanus* ATCC-1207	9α,11α-Epoxy-6β-hydroxypregn-4-ene-3,20-dione	[48]
17β-Acetoxy-A-nor-androst-4-en-3-one	*Rhizopus arrhizus* ATCC-11145	17β-Acetoxy-6β-hydroxy-A-nor-androst-4-en-3-one	[45]
17β-Acetoxy-B-homo-androsta-1,4-dien-3-one	*Rhizopus arrhizus* ATCC-11145	17β-Acetoxy-2β,6β,7aβ-trihydroxy-androst-4-en-3-one	[45]
17α,21-Dihydroxypregn-4-ene-3,20-dione	i) *Curvularia geniculata*	6β,17α-21-Trihydroxypregn-4-ene-3,20-dione	[49]
	ii) *Curvularia lunata*		
	iii) *Curvularia inaequalis*		
21-Acetoxy-17α-hydroxypregn-4-ene-3,20-dione	i) *Curvularia geniculata*	6β,17α,21-Trihydroxypregn-4-ene-3,20-dione	[49]
	ii) *Curvularia lunata*		
	iii) *Curvularia inaequalis*		
16α,17α-Epoxypregn-4-ene-3,20-dione	*Rhizopus strain* 80322	6β,11α-Dihydroxy-16α,17α-epoxypregn-4-ene-3,20-dione	[50]
Pregn-4-ene-3,20-dione	*Aspergillus phoenicis*	6β,11α-Dihydroxypregn-4-ene-3,20-dione	[51]

Androst-4-ene-3,17-dione	i) *Anabaena cylindrica* (IVL) ii) *Anacystis nidulans* (SVAL 1402-1) or iii) *Coelastrum proboscideum* var grucile (ATCC-30410) iv) *Scenedesmus quadricauda* (ATCC-11460)	6β-Hydroxyandrost-4-ene-3,17-dione	[52]
17β-Hydroxy-A-homo-5α-androstan-3-one	*Cunninghamella elegans*	6β,17β-Dihydroxy-A-homo-5α-androstan-3-one	[42]
17α-Hydroxypregn-4-ene-3,20-dione	*Bacillus* sp.	6β,17,20α-Trihydroxypregn-4-en-3-one	[41]
17α,21-Dihydroxypregn-4-ene-3,20-dione	*Bacillus* sp.	6β,17α,21-Trihydroxypregn-4-ene-3,20-dione	[53]
7aβ-Hydroxylation			
17β-Acetoxy-B-homoandrosta-1,4-dien-3-one	*Rhizopus arrhizus* ATCC-11145	i) 17β-Acetoxy-2β,7aβ-dihydroxyandrosta-4-en-3-one ii) 17β-Acetoxy-2β,6β,7aβ-trihydroxyandrost-4-en-3-one	[45]
7α-Hydroxylation			
22-Hydroxy-23,24-bisnorchol-4-en-3-one	i) *Botryodiplodia theobromae* ii) *Lasiodiplodia theobromae* iii) *Botryosphaeria* sp.	7α,22-Dihydroxy-23,24-bisnor-chol-4-en-3-one	[54]
17β-Hydroxy-A-nor-5α-androstan-2-one	*Cunninghamella elegans*	i) 7α,17β-Dihydroxy-A-nor-5α-androstan-2-one ii) 7α,9α,17β-Trihydroxy-A-nor-5α-androstan-2-one	[42]
5α-Androstane-3,17-dione	*Cunninghamella elegans*	i) 7α-Hydroxy-5α-androstane-3,17-dione ii) 7α,9α-Dihydroxy-5α-androstane-3,17-dione	[42]
17β-Hydroxy-A-homo-5α-androstan-3-one	*Cunninghamella elegans*	7α,17β-Dihydroxy-A-homo-5α-androstan-3-one	[42]
17β-Hydroxy-5α-androstan-3-one	*Cunninghmella elegans*	7α,17β-Dihydroxy-5α-androstan-3-one	[42]
D-Homo-5α-androstane-3,17-dione	*Cunninghamella elegans*	i) 7α-Hydroxy-D-homo-5α-androstane-3,17-dione ii) 7α,9α-Dihydroxy-D-homo-5α-androstane-3,17-dione	[43]
3β-Acetoxy-D-homo-5α-androstan-17a-one	*Cunninghamella elegans*	1β,3β,7α-Trihydroxy-D-homo-5α-androstan-17a-one	[43]
3β-Hydroxy-D-homo-5α-androstan-17a-one	*Cunninghamella elegans*	1β,3β,7α-Trihydroxy-D-5α-androstan-3,17a-one	[43]
D-Homo-5α-androstane-3,17a-dione	*Cunninghamella elegans*	i) 7α-Hydroxy-D-homo-5α-androstane-3,17a-dione ii) 7α,9α-Dihydroxy-D-homo-5α-androstane-3,17a-dione iii) 1β,7α-Dihydroxy-D-homo-5α-androstane-3,17a-dione	[43]
7β-Hydroxylation			
Taurolithocholic acid	*Mortierella ramanniana* Y2-1	Tauroursodeoxycholic acid	[55]
3α-Hydroxy-5β-cholanoic acid	*Fusarium equiseti* M-41	3α,7β-Dihydroxy-5β-cholanoic acid	[56]
Pregna-4,8(9)-diene-3,20-dione	*Rhizopus stolonifer* ATCC-6227b	7β-Hydroxypregna-4,8-diene-3,20-dione	[48]
3α-Hydroxy-5β-cholanoic acid	*Pleurotus ostreatus* IFO 30196	3α,7β-Dihydroxy-5β-cholanoic acid	[57]
3β-Hydroxy-D-homo-5α-androstan-17a-one	*Cunninghamella elegans*	3β,7β-Dihydroxy-D-homo-5α-androstan-17a-one	[43]
3β-Acetoxy-D-homo-5α-androstan-17a-one	*Cunninghamella elegans*	i) 3β,7β-Dihydroxy-D-homo-5α-androstan-17a-one ii) 1β,3β,7β-Trihydroxy-D-homo-5α-androstan	[43]
3β-Hydroxy-D-homo-5α-androstan-17a-one	*Cunninghamella elegans*	1β,3β,7β-Trihydroxy-D-homo-5α-androstan-17a-one	[43]
D-homo-5α-androstane-3,17a-dione	*Cunninghamella elegans*	1β,7β-Dihydroxy-D-homo-5α-androstane-3,17a-dione	[43]
9α-Hydroxylation			
Pregna-4,8(9)-diene-3,20-dione	*Curvularia lunata* NRRL-2380	9α,14α-Dihydroxypregn-4-ene,3,20-dione	[48]
Sitosterol	*Pseudomonas* NC1B 10590	9α-Hydroxyandrosta-1,4-diene-3,17-dione	[58]

Table 1. (*contd*)

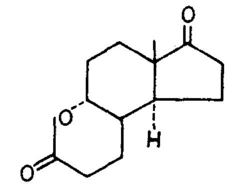
Substrate	Microorganism	Product	Reference
Sitosterol	*Mutant strain* T-21	i) 9-Hydroxypregna-4,17(20)-dien-3-one-20-carboxaldehyde ii) 9,22-Dihydroxy-23,24-dinor-chol-4-en-3-one	[38]
17β-Hydroxy-A-nor-5α-androstan-2-one	*Cunninghamella elegans*	i) 9α,17β-Dihydroxy-A-nor-5α-androstan-2-one ii) 7α,9α,17β-Trihydroxy-A-nor-5α-androstan-2-one	[42]
5α-Androstane-3,17-dione	*Cunninghamella elegans*	i) 9α-Hydroxy-5α-androstane-3,17-dione ii) 1β,9α-Dihydroxy-5α-androstane-3,17-dione iii) 7α,9α-Dihydroxy-5α-androstane-3,17-dione	[42]
17β-Hydroxy-A-homo-5α-androstan-3-one	*Cunninghamella elegans*	9α,17β-Dihydroxy-A-homo-5α-androstan-3-one	[42]
17β-Hydroxy-5α-androstan-3-one	*Cunninghamella elegans*	9α,17β-Dihydroxy-5α-androstan-3-one	[42]
D-Homo-5α-androstane-3,17-dione	*Cunninghamella elegans*	i) 7α,9α-Dihydroxy-D-homo-5α-androstane-3,17-dione ii) 9α,14α-Dihydroxy-D-homo-5α-androstane-3,17-dione	[43]
D-Homo-5α-androstane-3,17a-dione	*Cunninghamella elegans*	7α,9α-Dihydroxy-D-homo-5α-androstane-3,17a-dione	[43]
3β-Acetoxy-D-homo-5α-androstan-17-one	*Cunninghamella elegans*	3β,9α,14α-Trihydroxy-D-homo-5α-androstan-17-one	[43]
Sitosterol	*Mycobacterium fortuitum* NIO	9α-Hydroxyandrost-4-ene-3,17-dione	[59]
<u>10β-Hydroxylation</u>			
17β-Hydroxyestra-4,6-dien-3-one	*Rhizopus arrhizus* ATCC-11145	10β,17β-Dihydroxyestra-4,6-dien-3-one	[46]
<u>11α-Hydroxylation</u>			
Pregn-4-ene-3,20-dione	*Aspergillus ochraceus* (immobilized)	11α-Hydroxypregn-4-ene-3,20-dione	[60]
19-Nor-17β-hydroxyandrost-4-en-3-one	*Aspergillus ochraceus* ATCC-1008 and *Arthrobacter simplex* ATCC-6946 (Mixed culture)	3,11α-Dihydroxyestra-1,3,5(10)-trien-17-one	[61]
16α,17α-Epoxypregn-4-en-3,20-dione	*Rhizopus* 80322	16α,17α-Epoxy-11α-hydroxypregn-4-ene-3,20-dione	[62]
17β-Acetoxyandrost-4-en-3-one	*Rhizopus arrhizus* ATCC-11145	17β-Acetoxy-11α-hydroxyandrost-4-en-3-one	[46]
17α,21-Dihydroxypregn-4-ene-3,20-dione	*Beauveria bassiana*	11α,17α,21-Trihydroxypregn-4-ene-3,20-dione	[63]
16β,17β-Epoxypregn-4-ene-3,20-dione	*Rhizopus nigricans* (immobilized)	16β,17β-Epoxy-11α-hydroxypregn-4-ene-3,20-dione	[64]
Pregn-4-ene-3,20-dione	*Aspergillus ochraceus G*-8 (immobilised)	11α-Hydroxypregn-4-ene-3,20-dione	[65]
Pregn-4-ene-3,20-dione	*Aspergillus ochraceus*	i) 11α-Hydroxypregn-4-ene-3,20-dione ii) 6β,11α-Dihydroxypregn-4-ene-3,20-dione	[47]
Pregn-4-ene-3,20-dione	*Colletotrichum antirrhini* SC-2144	11α-Hydroxypregn-4-ene-3,20-dione	[66]
Pregn-4-ene-3,20-dione	*Rhizopus stolonifer* ATCC 6227b	11α-Hydroxypregn-4-ene-3,20-dione	[48]
17β-Hydroxyandrosta-1,4-dien-3-one	*Rhizopus arrhizus* ATCC-11145	11α,17β-Dihydroxyandrosta-1,4-dien-3-one	[45]
17β-Acetoxyandrosta-1,4-dien-3-one	*Rhizopus arrhizus* ATCC-11145	17β-Acetoxy-11α-hydroxyandrosta-1,4-dien-3-one	[45]
Androsta-1,4-diene-3,17-dione	*Rhizopus arrhizus* ATCC-11145	11α-Hydroxyandrosta-1,4-diene-3,17-dione	[45]

17β-Hydroxyandrost-4-en-3-one	*Rhizopus nigricans*	11α,17β-Dihydroxyandrost-4-en-3-one	[67]
17β-Methoxyandrost-4-en-3-one	*Rhizopus nigricans*	11α-Hydroxy-17β-methoxyandrost-4-en-3-one	[67]
Pregn-4-en-3,20-dione	*Aspergillus phoenicis* (immobilized)	11α-Hydroxypregn-4-ene-3,20-dione	[68]
17α,21-Dihydroxypregn-4,-ene-3,20-dione	*Cylindrocladium simplex*	11α,17α,21-Trihydroxypregn-4-ene-3,20-dione	[69]
17α,21-Dihydroxypregn-4-ene-3,20-dione	*Cunninghamella elegans*	11α,17α,21-Trihydroxypregn-4-ene-3,20-dione	[70]
17α,21-Dihydroxypregn-4-ene-3,20-dione	i) *Cunninghamella blakesleeana* ii) *Cunninghamella echniulata* iii) *Cunninghamella homothallica* iv) *Cunninghamella japonica*	11α,17α,21-Trihydroxypregn-4-ene-3,20-dione	[49]
21-Acetoxy-17α-hydroxypregn-4-ene-3,20-dione	i) *Cunninghamella blakesleeana* ii) *Cunninghamella echniulata* iii) *Cunninghamella homothallica* iv) *Cunninghamella japonica*	21-Acetoxy-11α,17α-dihydroxypregn-4-ene-3,20-dione	[49]
17α,21-Dihydroxypregn-4-ene-3,20-dione	i) *Curvularia geniculata* ii) *Curvularia lunata* iii) *Curvularia inaequalis*	11α,17α,21-Trihydroxypregn-4-ene-3,20-dione	[49]
21-Acetoxy-17α-hydroxypregn-4-ene-3,20-dione	i) *Curvularia geniculata* ii) *Curvularia lunata* iii) *Curvularia inaequalis*	11α,17α,21-Trihydroxypregn-4-ene-3,20-dione	[49]
Pregn-4-ene-3,20-dione	*Aspergillus phoenicis*	i) 11α-Hydropregn-4-ene-3,20-dione ii) 6β,11α-Dihydroxypregn-4-ene-3,20-dione	[51]
5α-Androstane-3,17-dione	*Aspergillus ochraceus*	11α-Hydroxy-5α-androstane-3,17-dione	[42]
A-Homo-5α-androstane-3,17-dione	*Aspergillus ochraceus*	11α-Hydroxy-A-homo-5α-androstane-3,17-dione	[42]
17β-Hydroxy-A-nor-5α-androstan-2-one	*Cunninghamella elegans*	11α,17β-Dihydroxy-A-nor-5α-androstan-2-one	[42]
17β-Hydroxy-A-homo-5α-androstan-3-one	*Cunninghamella elegans*	11α,17β,-Dihydroxy-A-homo-5α-androstan-3-one	[42]
Pregn-4-ene-3,20-dione	*Aspergillus ochraceus* (immobilized)	11α-Hydroxypregn-4-ene-3,20-dione	[26]
3β-Acetoxy-D-homo-5α-androstan-17a-one	*Aspergillus ochraceus*	3β,11α-Dihydroxy-D-homo-5α-androstan-17a-one	[43]
17β-Acetoxy-A-norandrost-4-en-3-one	*Rhizopus arrhizus* ATCC-11145	17β-Acetoxy-11α-hydroxy-A-nor-androst-4-en-3-one	[45]
16α,17α-Epoxypregn-4-ene-3,20-dione	*Rhizopus* strain 80322	i) 16α,17α-Epoxy-11α-hydroxypregn-4-ene-3,20-dione ii) 6β,11α-Dihydroxy-16α,17α-epoxypregn-4-ene-3,20-dione	[50]
Pregn-4-ene-3,20-dione	*Cunninghamella echinulata*	i) 11α-Hydroxypregn-4-ene-3,20-dione ii) 11α,17α-Dihydroxypregn-4-ene-3,20-dione	[71]
17α-Hydroxy-16α-methylpregna-1,4-diene-3,20-dione	*Absidia glauca*	11α,17α-Dihydroxy-16α-methylpregna-1,4-diene-3,20-dione	[72]
21-Acetoxy-17α-hydroxy-16α-methylpregn-4-ene-3,20-dione	*Beauveria bassiana* AS 69	16α-Methyl-11α,17α,21-trihydroxypregn-4-ene-3,20-dione	[73]
<u>11β-Hydroxylation</u>			
Pregna-1,4-dien-3-one-20-carboxylic acid	*Cochliobolus lunatus* m118	11β-Hydroxypregna-1,4-dien-3-one-20-carboxylic	[74]
17α,21-Dihydroxypregn-4-ene-3,20-dione	i) *Pellicularia filamentosa* microsclerotia IFO 6298 ii) *Pellicularia filamentosa* sasakii IFO 5254	11β,17α,21-Trihydroxypregn-4-ene-3,20-dione	[75]

Table 1. (*contd*)

Substrate	Microorganism	Product	Reference
17α,21-Dihydroxypregn-4-ene-3,20-dione	*Curvularia lunata* and *Arthrobacter simplex* (*immobilized*)	11β,17α,21-Trihydroxypregna-1,4-diene-3,20-dione	[23]
21-*Acetoxy*-17α-hydroxypregn-4-ene-3,20-dione	*Absidia orchidis*	11β,17α,21-Trihydroxypregn-4-ene-3,20-dione	[76]
17β-Hydroxyestra-4,9(10)-dien-3-one	*Curvularia lunata* NRRL-2380	11β,17β-Dihydroxyestra-4,9(10)-dien-3-one	[48]
17α,20β,21-Trihydroxypregn-4-en-3-one	*Curvularia lunata*	11β,17α,20β,21-Tetrahydroxypregn-4-en-3-one	[77]
17α,21-Dihydroxypregn-4-ene-3,20-dione	i) *Cunninghamella blakesleeana* ii) *Cunninghamella echniulata* iii) *Cunninghamella homothallica* iv) *Cunninghamella japonica*	11β,17α,21-Trihydroxypregn-4-ene-3,20-dione	[49]
21-Acetoxy-17α-hydroxypregn-4-ene-3,20-dione	i) *Cunninghamella blakesleeana* ii) *Cunninghamella echniulata*	21-Acetoxy-11β,17α-dihydroxypregn-4-ene-3,20-dione	[49]
21-Acetoxy-17α-hydroxypregn-4-ene-3,20-dione	i) *Cunninghamella homothallica* ii) *Cunninghamella japonica*	21-Acetoxy-11β,17α-dihydroxypregn-4-ene-3,20-dione	[49]
17α,21-Dihydroxypregn-4-ene-3,20-dione	i) *Curvularia geniculata* ii) *Curvularia lunata* iii) *Curvularia inaequalis*	11β,,17α,21-Trihydroxypregn-4-ene-3,20-dione	[49]
21-Acetoxy-17α-hydroxypregn-4-ene-3,20-dione	i) *Curvularia geniculata* ii) *Curvularia lunata* iii) *Curvularia inaequalis*	11β,17α,21-Trihydroxypregn-4-ene-3,20-dione	[49]
17α,21-Dihydroxypregn-4-ene-3,20-dione	*Curvularia lunata* BKM F-644	11β,17α,21-Trihydroxypregn-4-ene-3,20-dione	[78]
21-Acetoxy-17α-hydroxypregn-4-ene-3,20-dione	*Curvularia lunata* BKM F-644	11β,17α,21-Trihydroxypregn-4-ene-3,20-dione	[78]
12α-Hydroxylation			
17β-Hydroxy-5α-androstan-3-one	*Cunninghamella elegans*	12α,17β-Dihydroxy-5α-androstan-3-one	[42]
12β-Hydroxylation			
Pregna-1,4-dien-3-one-20-carboxylic acid	*Cochliobolus lunatus* m 118	12β-Hydroxypregna-1,4-diene-3-one-20-carboxylic acid	[74]
17β-Hydroxy-A-nor-5α-androstan-2-one	*Cunninghamella elegans*	12β-Hydroxy-A-nor-5α-androstane-2,17-dione	[42]
14-Hydroxylation			
Pregn-4-ene-3,20-dione	*Collectotrichum antirrhini* SC-2144	i) 14α-Hydroxyandrosta-1,4-diene-3,17-dione ii) 14α-Hydroxypregn-4-ene-3,20-dione iii) 14α-Hydroxypregna-1,4-diene-3,20-dione	[66]
Pregna-4,8(9)-diene-3,20-dione	*Curvularia lunata* NRRL-2380	9α,14α-Dihydroxypregn-4-ene-3,20-dione	[48]
17α,21-Dihydroxypregn-4-ene-3,20-dione	i) *Curvularia geniculata* ii) *Curvularia lunata* iii) *Curvularia inaequalis*	14α,17α,21-Trihydroxypregn-4-ene-3,20-dione	[49]
17α,21-Dihydroxypregn-4-ene-3,20-dione	*Bacillus* sp.	i) 14α-Hydroxyandrost-4-ene-3,17-dione ii) 14α,17α,21-Trihydroxypregn-4-ene-3,20-dione	[53]
21-Acetoxy-17α-hydroxypregn-4-ene-3,20-dione	i) *Curvularia geniculata* ii) *Curvularia lunata* iii) *Curvularia inaequalis*	14α,17α,21-Trihydroxypregn-4-ene-3,20-dione	[49]

Androst-4-ene-3,17-dione	i) *Coelastrum proboscideum var grucile* (ATCC-30410) ii) *Scenedesmus quadricauda* ATCC-11460	14α-Hydroxyandrost-4-ene-3,17-dione	[52]
Androst-4-ene-3,17-dione	i) *Ankistrodesmus angustus* ATCC-30448 ii) *Scenedesmus quadricauda* ATCC-11460	14α,17β-Dihydroxyandrost-4-en-3-one	[52]
17β-Hydroxyandrost-4-en-3-one	i) *Coelastrum proboscideum var grucile* (ATCC-30410) ii) *Scenedesmus quadricauda* ATCC-11460	14α,17β-Dihydroxyandrost-4-en-3-one	[52]
17β-Hydroxy-5α-androstan-3-one	*Cunninghamella elegans*	1β,14α,17β-Trihydroxy-5α-androstan-3-one	[42]
D-Homo-5α-androstane-3,17-dione	*Cunninghamella elegans*	9α,14α-Dihydroxy-D-homo-5α-androstane-3,17-dione	[43]
3β-Acetoxy-D-homo-5α-androstan-17-one	*Cunninghamella elegans*	3β,9α,14α-Trihydroxy-D-homo-5α-androstan-17-one	[43]
<u>15α-Hydroxylation</u>			
Estra-4,9-diene-3,17-dione	*Fusarium oxysporum*	15α-Hydroxyestra-4,9-diene-3,17-dione	[79]
Androst-4-ene-3,17-dione	i) *Fusarium* sp. ii) *Penicillium stoloniferum* CBS P102	15α-Hydroxyandrost-4-ene-3,17-dione	[80]
17α-Hydroxypregn-4-ene-3,20-dione	*Bacillus* sp.	15α,17α-Dihydroxypregn-4-ene-3,20-dione	[41]
17α,21-Dihydroxypregn-4-ene-3,20-dione	*Bacillus* sp.	15α,17α,21-Trihydroxypregn-4-ene-3,20-dione	[53]
<u>15β-Hydroxylation</u>			
3α-Hydroxy-5β-cholanoic acid	*Cunninghamella* sp. ST-22	3α,15β-Dihydroxy-5β-cholanoic acid	[81]
17β-Hydroxyandrost-4-en-3-one	*Aspergillus fumigatus*	15β,17β-Dihydroxyandrost-4-en-3-one	[82]
3α-Hydroxy-5β-cholanoic acid	*Cunninghamella blakesleeana* ST-22	3α,15β-Dihydroxy-5β-cholanoic acid	[36]
3α-Hydroxy-5β-cholanoic acid	*Cunninghamella echniulata* ST-22	3α,15β-Dihydroxy-5β-cholanoic acid	[83]
<u>16α-Hydroxylation</u>			
9α-Fluoro-11β,17α,21-trihydroxypregn-4-ene-3,20-dione	*Streptomyces roseochromogenes* (immobilized)	9α-Fluoro-11β,16α,17α,21-tetrahydroxypregn-4-ene-3,20-dione	[84]
Pregn-4-ene-3,20-dione	*Streptomyces coriofaciens*	16α-Hydroxypregn-4-ene-3,20-dione	[85]
<u>17α-Hydroxylation</u>			
Pregn-4-ene-3,20-dione	*Cunninghamella echinulata*	i) 17α-Hydroxypregn-4-ene-3,20-dione ii) 11α,17α-Dihydroxypregn-4-ene-3,20-dione	[71]
<u>19-Hydroxylation</u>			
17α,21-Dihydroxypregn-4-ene-3,20-dione	i) *Pellicularia filamentosa microsclerotia* IFO 6298 ii) *Pellicularia filamentosa sasakii* IFO 5254	17α,19,21-Trihydroxypregn-4-ene-3,20-dione	[75]
<u>22-Hydroxylation</u>			
Sitosterol	Mutant strain T-21	9,22-Dihydroxy-23,24-dinor-chol-4-en-3-one	[38]
<u>26-Hydroxylation</u>			
Sitosterol	*Pseudomonas* NCIB 10590	26-Hydroxy-24-ethylcholest-4-en-3-one	[58]



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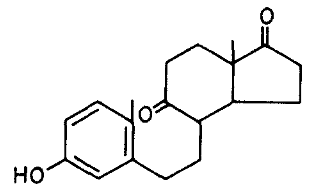

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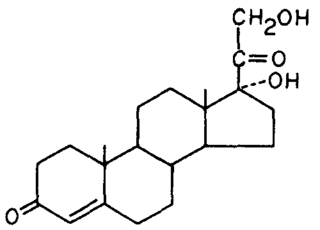

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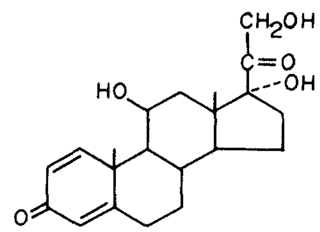

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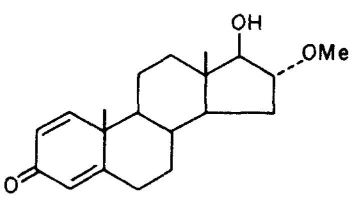

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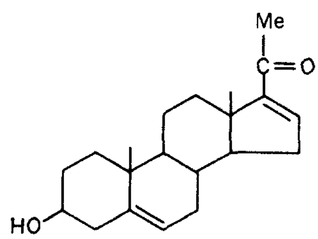

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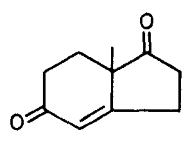

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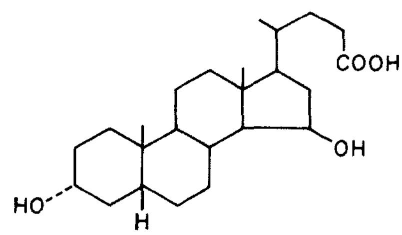

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tional period for the transformation has been reported to be 25 days. A new technique involving the use of an airlift loop reactor for the transformation of steroids by immobilized cells has been published [24]. The flow behaviour of calcium alginate beads in an airlift reactor with an external loop was dependent on the airflow rate into and the amounts of beads in the reactor. The performance of immobilized *Arthrobacter simplex* for the Δ^1-dehydrogenation of hydrocortisone in the airlift reactor was comparable to that in a stirred tank reactor. The stability of the calcium alginate beads was found by the investigators to be significantly greater in the airlift reactor. A technique of immobilization of cells by adhesion on glass, either by coating the support with colloidal particles of hydrous alumina or by pretreating the cells with aluminium has been described by Mozes *et al.* [25]. The use of glass slides as a model support has shown that a single, dense and regular layer of immobilized cells is achieved. The quantity of immobilized cells was estimated to be $\sim 7 \times 10^7$ cells/cm^2. Immobilization on glass beads or glass wool packed as a bed in a column was also successful. The investigators tested the transformation of cortisol to prednisolone and observed that the specific activity of immobilized cells was not different from that of free cells. The use of a microreactor with the immobilized bacteria as biocatalyst demonstrated the feasibility of repeated use of the microorganisms. Bihari *et al.* [26] reported the use of fluidized-bed reactor for the transformation of steroids by immobilized cells. The spores of *Aspergillus ochraceus* were immobilized in a fluidized-bed column reactor for 11α-hydroxylation of progesterone. The performance of the reactor was found to be favourable for employment of such a system for steroid transformation. The column retained 60 % of its initial activity after 24 days of use. A continuous transformation involving sterol side-chain cleavage by immobilized *Moraxella* species has recently been reported [27]. Cholesteryl sulphate was transformed to dehydroepiandrosterone sulphate and 19-hydroxycholesteryl acetate to estrone. The physical and chemical methods of immobilization have been compared.

Other newer techniques for selective transformation

Wolf and Kominek [28] have reported a novel method for improved steroid Δ^1-dehydrogenation using heat-dried bacterial cells. The steroid 1-dehydrogenase was stable at high temperature and other enzymes were inactivated. Thus Δ^1-steroids were produced by heat-dried *Arthrobacter simplex* cells. The highest rates of 1-dehydrogenation (>97 %) were observed for cells dried at 40–60°. The use of heat-dried *A. simplex* provided several advantages of yield and simplicity. The method of transposon mutagenesis has been used for the production of therapeutically useful steroids in good yield from bile acids [29]. The high yields are obtained by inactivation of steroid degrading enzymes by the introduced mutation. For example, a plasmid coding for the Kanamycin resistant transposon Tn 5 was introduced into *Pseudomonas putida* ATCC 31752, and the resulting mutant was cultivated in medium containing Kanamycin and cholic acid (2g/l). Among 1012 clones obtained, two were blocked in 12α-OH→12β-OH epimerization, three in α-hydroxylation and five in 9,10-*seco*-1,3,5(10)-androstatriene-9,17-dione degradation. One of the epimerization-blocked mutants was used to convert sodium cholate to 7α,12α-dihydroxy-1,4-androstadiene-3,17-dione in *ca* 70 % yield. Improvement in the yield of prednisolone from hydrocortisone by *A. simplex* using inhibitors has been reported [30]. Apart from Δ^1-dehydrogenase *A. simplex* contained another enzyme, 20β-ketoreductase which reduced hydrocortisone and prednisolone in the culture. The use of iodoacetate and *p*-chloromercuric benzoic acid inhibited the 20β-keto-reductase without affecting the Δ^1-dehydrogenase activity. The yield of prednisolone was improved with the addition of inhibitors and selection of medium. The kinetics of the two enzymes have also been described.

A very high degree of steroid transformation by *Flavobacterium dehydrogenans* in two-liquid-phase system has recently been described [31]. The production of 4-androstene-3,17-dione by *F. dehydrogenans* from androstenolone acetate in octane culture (vol/vol = 1:1) two-liquid-phase system was compared with the conversion rate in a Tween 80 containing medium commonly used in industry and in a culture where no organic solvent or detergent was added. The rate of 4-androstene-3,17-dione production at the optimal cell density in the two-liquid-phase system and Tween 80 containing medium were about 6 and 1.5 times as high respectively as in the aqueous medium. A very high degree of conversion (>98 %) could be reached in the two-liquid-phase system which could not be obtained in aqueous medium. An easy way to recover the product in high purity has also been proposed.

The mathematical model described for crystallization and transformation of steroids by *Arthrobacter globiformis* [32] was successfully used for transformation of hydrocortisone and prednisolone. The experimental results were in good agreement with theoretical values.

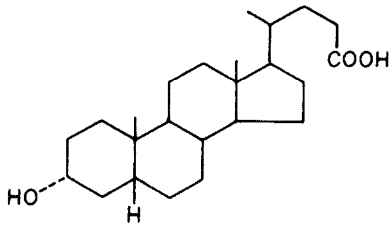
Novel microbial reactions and new metabolites

A novel microbial transformation of dehydrocholic acid by *Streptomyces gelaticus* into steroid dimers containing a benzene ring has been reported by Nakao *et al.* [33]. The microbial production of a novel androstane analogue, 16α-methoxy-17β-hydroxyandrosta-1,4-dien-3-one (**13**) by fermentation of 16-dehydropregnenolone (**14**) with a strain of *Arthrobacter simplex* (IICB-321) has been described [21, 34]. Thus microbial generation of a methoxy group involving double bond transformation in a steroid molecule has appeared for the first time. The mechanism of formation of metabolite (**13**) from compound (**14**) has also been discussed. Selective transformation of sitosterol to (7a *S*)-2,3,7,7a-tetrahydro-7a-methyl-(1H)-indene-1,5-(6H)-dione (**15**) by *Mycobacterium fortuitum* NRRL B-15026 has been patented [35]. The strain was obtained by mutagenesis of *M. fortuitum* ATCC 6842 with nitrosoguanidine.

A new bile acid metabolite, 3α,15β-dihydroxy-5β-cholanic acid (**16**) is produced in good yield by transformation of lithocholic acid (**17**) by the fungus, *Cunninghamella blakesleeana* ST-22 [36]. Hydrophobicity measurements and *in vitro* cholesterol solubilization tests showed that the metabolite (**16**) was as effective as ursodeoxycholic acid (**18**) in cholesterol solubilization. Knight and Wovcha [37] reported the isolation of methyl 3-hydroxy-9-oxo-9,10-seco-23,24-dinor-1,3,5(10)-cholatrienoate (**19**) by microbial degradation of cholesterol and sitosterol by a mutant (UC 9778) of *Mycobacterium fortuitum* ATCC 6842. These authors also described the isolations of the interesting metabolites, 9-hydroxy-3-oxo-23,24-dinor-4,17(20)-choladien-22-al (**20**) and 9,22-dihydroxy-23,24-dinor-4-cholen-3-one (**21**) from a sitosterol bioconversion [38]. A transposon mutant strain from *Pseudomonas* species has been reported to be used for the production of a yellow muconic like derivative, tentatively characterized as 3,7-dihydroxy-5,9,17-trioxo-4(5),9(10)-diseco-androsta-1(10),2-dien-4-oic acid (**22**) [39]. Two new pregnane analogues, 14-hydroxy-5α-pregnane-3,6,20-trione (**23**) and 11α,14-dihydroxy-4-pregnene-3,20-dione (**24**) of potential therapeutical interest have been reported to have been produced as metabolities of progesterone by a *Bacillus* sp. [40]. Fermentation of 17-hydroxyprogesterone with the same strain also yielded a new pregnane analogue, 6β,17,20α-trihydroxypregn-4-en-3-one (**25**) [41].

Future possibilities

Further developmental activities relating to controlled multiple transformations for the purpose of cost reduction are expected. Encouraging results in ordered sequential fermentations with vegetative cells have already been obtained. There is distinct possibility of further developments on conducting two or more microbial steps in one fermentation using mixed cultures. In mixed fermentations it is essential that the two organisms are capable of inducing the desired enzymes in one another's presence. Development of new steroids of useful biological activity requiring new biotechnology may receive greater attention. Continuing attempts are expected for utilization of widely available sterols obtained as industrial by-



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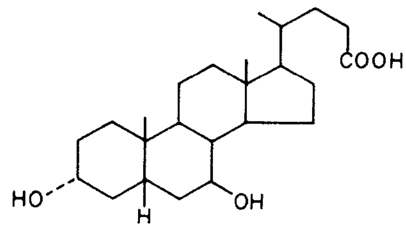

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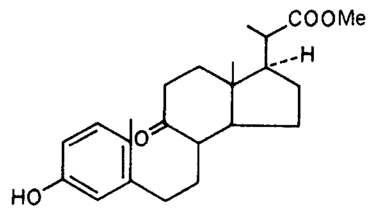

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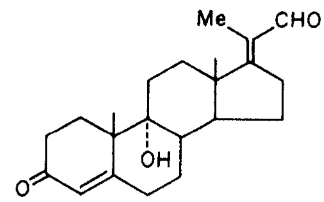

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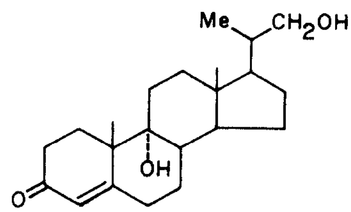

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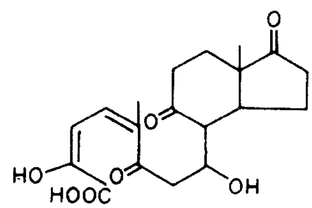

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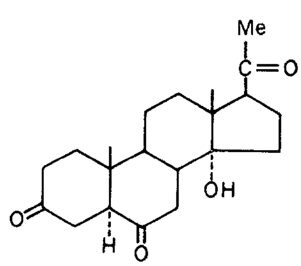

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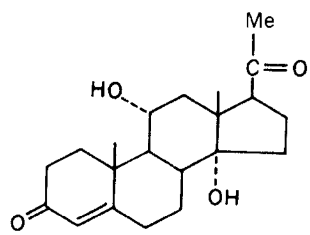

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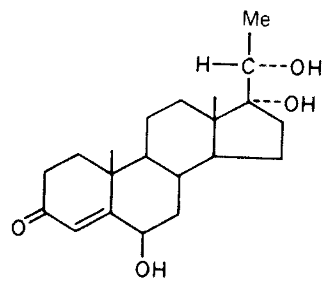

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products in the production of useful steroid intermediates. Greater emphasis on the applications of the newer concepts of genetic engineering of microorganisms for their improvement as steroid transforming agents or as agents for *de novo* steroid biosynthesis is anticipated. In fact, as already described, a few applications of transposon mutagenesis for improvement of strains in steroid transformations have already been reported.

Tables

A compilation of microbial hydroxylations of various steroid substrates is presented in Table 1. The hydroxylation reactions have been arranged according to increasing order of the carbon atom on which hydroxylation has taken place. Table 2 shows the side chain degradations achieved, and dehydrogenations are pre-

Table 2. Side chain degradation

Substrate	Microorganism	Product	Reference
3α-Hydroxy-5β-cholanoic acid	*Pseudomonas* NCIB 10590	i) Androsta-1,4-diene-3,17-dione ii) 17β-Hydroxyandrost-4-en-3-one iii) 17β-Hydroxyandrosta-1,4,dien-3-one iv) 3-Oxopregn-4-ene-20-carboxylic acid v) 3-Oxopregna-1,4-diene-20-carboxylic acid	[86]
5β-Cholanoic acid-3α-sulphate	*Pseudomonas* NCIB 10590	i) Androsta-1,4-diene-3,17-dione ii) 3-Oxopregna-1,4-diene-20-carboxylic acid	[87]
3α,6α-Dihydroxy-5β-cholanoic acid	*Pseudomonas putida* ATCC 31752	i) 3,9β-Dihydroxy-6ξ, 9α-epoxy-9,10-*seco*-1,3,5 (10)-androstatrien-17-one ii) 3-Hydroxy-9β-methoxy-6ξ,9α-epoxy-9,10-*seco*-1,3,5(10)-androstatrien-17-one	[88]
3α,7α-Dihydroxy-5β-cholanoic acid	*Pseduomonas putida* ATCC 31752	3,7ξ-Dihydroxy-9,10-*seco*-1,3,5(10)-androstatriene-9,17-dione	[88]
3α,11β-Dihydroxy-5β-cholanoic acid	*Alcaligenes faecalis* FERM BP-204	11β-Hydroxypregn-4-en-3-one-20-carbaldehyde	[17]
3α,12α-Dihydroxy-5β-cholanoic acid	*Pseudomonas putida* D 4014-A 1615	12β-Hydroxyandrost-4-ene-3,17-dione	[89]
3α,12α-Dihydroxy-5β-cholanoic acid	i) *Pseudomonas* sp. mutant RAL 1.22 ii) *Pseudomonas* sp. mutant RAL 8.15 iii) *Pseudomonas* sp. mutant RAL 91.11	12β-Hydroxyandrosta-1,4-diene-3,17-dione	[39]
3α,12α-Dihydroxy-5β-cholanoic acid	i) *Pseudomonas* sp. mutant RAL 8.23 ii) *Pseudomonas* sp. mutant RAL 8.24 iii) *Pseudomonas* sp. mutant RAL 20.5	12α-Hydroxyandrosta-1,4-diene-3,17-dione	[39]
3α,12α-Dihydroxy-5β-cholanoic acid	*Pseudomonas* sp. mutant RAL 8.14	i) 12α-Hydroxyandrosta-1,4-diene-3,17-dione ii) 12α-Hydroxy-3-oxo-23,24-dinorchola-1,4-dien-22-oic acid	[39]
3α,12α-Dihydroxy-5β-cholanoic acid	i) *Pseudomonas* sp. mutant RAL 1.25 ii) *Pseudomonas* sp. mutant RAL 8.16 iii) *Pseudomonas* sp. mutant RAL 20.8	3,12β-Dihydroxy-9,10-secoandrosta-1,3,5(10)-triene-9,17-dione	[39]
3α,7α-Dihydroxy-5β-cholanoic acid	*Pseudomonas* sp. mutant RAL 8.23	3,7-Dihydroxy-4,5,9,10-diseco-5,9,17-trioxoandrosta-1(10),2-dien-4-oic acid	[39]
3α-Hydroxy-5β-cholanoic acid	*Corynebacterium* 320-6-2-1 (FERM P-7521)	i) 3-Oxopregna-1,4-diene-20-carboxylic acid	[90]

Table 2. (*contd*)

Substrate	Microorganism	Product	Reference
		ii) 3-Oxopregna-1,4,17(20)-triene-20-carboxylic acid iii) Androsta-1,4-diene-3,17-dione	
3α,12α-Dihydroxy-5β-cholanoic acid	*Alcaligenes faecalis* D 4020-K15	12α-Hydroxypregna-1,4-dien-3-one-20-carbaldehyde	[16]
3α,12α-Dihydroxy-5β-cholanoic acid	*Pseudomonas putida*	12α-Hydroxyandrosta-1,4-diene-3,17-dione	[91]
3α,12α-Dihydroxy-5β-cholanoic acid	*Alcaligenes faecalis*	12α-Hydroxypregna-1,4-dien-3-one-20-carboxylic acid	[92]
3α,12α-Dihydroxy-5β-cholanoic acid	*Pseudomonas putida* ATCC-31752	3,12β-Dihydroxy-9,10-*seco*-1,3,5(10)-androstatriene-9,17-dione	[88]
3α,7α,12α-Trihydroxy-5β-cholanoic acid	i) *Pseudomonas putida* ATCC-31752 ii) *Pseudomonas* sp. ATCC-31753	7α,12α-Dihydroxyandrosta-1,4-diene-3,17-dione	[93]
3α,7α,12α-Trihydroxy-5β-cholanoic acid sodium salt	*Pseudomonas putida* ATCC-31752	7α,12α-Dihydroxyandrosta-1,4-diene-3,17-dione	[29]
3α,7α,12α-Trihydroxy-5β-cholanoic acid	*Pseudomonas putida* ATCC-31752	3,7ξ,12β-Trihydroxy-9,10-*seco*-1,3,5(10)-androsta-triene-9,17-dione	[88]
Cholestane	i) *Pseudomonas stutzeri* ii) *Micrococcus* sp. iii) *Cephalosporium longisporum*	5α-Androstan-17-one	[94]
i-Cholestanone	*Moraxella* sp.	3α,5α-Cycloandrostane-6,17-dione	[94]
Cholestanol	*Moraxella* sp.	5α-Androstane-3,17-dione	[94]
Cholesterol	*Pseudomonas convexa*	3β-Hydroxyandrost-5-en-17-one	[94]
Cholesterol	i) *P. stutzeri* ii) *Micrococcus* sp. iii) *Cephalosporium longisporum*	Androst-4-ene-3,17-dione	[94]
Cholesterol	*P. stutzeri*, *Micrococcus* sp. and *C. longisporum* (mixed culture)	i) 3β-Hydroxyandrost-5-en-17-one ii) Androst-4-ene-3,17-dione	[94]
Cholesterol	*Moraxella* sp.	i) Androst-4-ene-3,17-dione ii) Androsta-1,4-diene-3,17-dione	[94]
Cholesterol	*Arthrobacter* sp. 317	i) Androsta-1,4-diene-3,17-dione ii) Androst-4-ene-3,17-dione	[95]
Cholesterol	*Rhodococcus australis* CSIR-236.457	3aα-H-4α-(3′-propionic acid)-5α-hydroxy-7aβ-methylhydroindan-1-one-δ-lactone	[20]
Cholesterol	*Mycobacterium* CCM 3528	22-Hydroxy-23,24-bisnorchola-1,4-dien-3-one	[15]
Cholesterol	*Mycobacterium* CCM 3529	Androsta-1,4-diene-3,17-dione	[15]
Cholesterol	*Corynebacterium* 320-6-2-2	i) Pregn-4-en-3-one-20-carboxylic acid ii) Pregna-1,4-dien-3-one-20-carboxylic acid iii) Androsta-1,4-diene-3,17-dione	[13]

Cholesterol	i) *Mycobacterium* NRR1 B 3805 ii) *Mycobacterium phlei* (MYC-4)	17β-Hydroxyandrost-4-en-3-one	[18]
Cholesterol	*Mycobacterium phlei* (MYC-4)	17β-Hydroxyandrosta-1,4-dien-3-one	[18]
Cholesterol	*Mycobacterium* CCM-3529	i) Androsta-1,4-diene-3,17-dione ii) Methyl-3-oxo-23,24-bisnor-1,4-choladienoate iii) Androst-4-ene-3,17-dione iv) 22-Hydroxy-23,24-bisnor-chola-1,4-dien-3-one v) 17β-Hydroxyandrost-4-en-3-one vi) 17β-Hydroxyandrosta-1,4-dien-3-one	[96]
Cholesterol	*Pseudomonas* NCIB 10590	Androsta-1,4-diene-3,17-dione	[97]
Cholesterol	*Rhodococcus australis*	3aα-H-4α-(3′-propionic acid)-5α-hydroxy-7aβ-methylhexahydro-1-indanone-δ-lactone	[98]
Cholesterol	*Mycobacterium fortuitum* ATCC-6842 mutant (UC-9778)	Methyl-3-hydroxy-9-oxo-9,10-*seco*-23,24-dinor-1,3,5(10)-cholatrienoate	[37]
Cholesteryl acetate	*Moraxella* sp.	i) Androst-4-ene-3,17-dione ii) Androsta-1,4-diene-3,17-dione	[94]
3β-Chlorocholest-5-ene	*Moraxella* sp.	3β-Cholroandrost-5-en-17-one	[94]
3β-Methoxycholest-5-ene	*Moraxella* sp.	3β-Methoxyandrost-5-en-17-one	[94]
Potassium cholesteryl sulphate	*Moraxella* sp.	Potassium dehydroepiandrosterone sulphate	[94]
Potassium cholesteryl sulphate	*Moraxella* sp. (immobilized)	Dehydroeipandrosterone sulphate	[94]
Potassium cholesteryl sulphate	*Moraxella* sp. (immobilized)	Dehydroepiandrosterone sulphate	[27]
19-Hydroxycholesteryl acetate	*Moraxella* sp. (immobilized)	Estrone	[27]
3β-Acetoxycholest-5-en-19-ol	*Moraxella* sp. (immobilized)	Estrone	[94]
Cholest-5-en-3β-ol-hydrogen succinate	*Arthrobacter* sp. 317	i) Androsta-1,4-diene-3,17-dione ii) Androst-4-ene-3,17-dione	[95]
3β-Carbamoyloxy-5-cholestene	*Mycobacterium phlei* MNG-29	3β-Carbamoyloxy-5-androsten-17-one	[99]
Cholest-4-en-3-one	*P. stutzeri*, *Micrococcus* sp. and *C. longisporum* (mixed culture)	i) Androst-4-ene-3,17-dione ii) 3β-Hydroxyandrost-5-en-17-one	[94]
Pregn-4-ene-3,20-dione	*Arthrobacter* sp. 317	i) Androsta-1,4-diene-3,17-dione ii) Androst-4-ene-3,17-dione	[95]
Pregn-4-ene-3,20-dione	*Colletotrichum antirrhini* Sc-2144	i) Androst-4-ene-3,17-dione ii) Androsta-1,4-diene-3,17-dione iii) 14α-Hydroxyandrosta-1,4-diene-3,17-dione	[66]
Pregn-4-ene-3,20-dione	*Trichurus spiralis*	i) Testololactone ii) 17β-Hydroxyandrost-4-en-3-one iii) Androst-4-ene-3,17-dione iv) Androsta-1,4-diene-3,17-dione	[100]
Pregn-4-ene-3,20-dione	*Arthrobacter simplex* (IICB-321)	i) 17β-Hydroxyandrosta-1,4-dien-3-one ii) Androsta-1,4-diene-3,17-dione iii) 3-Hydroxy-9,10-*seco*-1,3,5(10)-androstatriene-9,17-dione iv) 3aα-H-4α-(3′-propionic acid)-5α-hydroxy-7aβ-methylhexahydro-1-indanone-δ-lactone	[21]
Pregna-4,8(9)-diene-3,20-dione	*Rhizopus stolonifer* ATCC 6227 b	9α,11α-Epoxyandrosta-1,4-diene-3,17-dione	[48]

Table 2. (*contd*)

Substrate	Microorganism	Product	Reference
17α,21-Dihydroxypregn-4-ene-3,20-dione	*Bacillus* sp.	i) Androst-4-ene-3,17-dione ii) 14-Hydroxyandrost-4-ene-3,17-dione	[53]
11β,17α,21-Trihydroxy-pregn-4-ene-3,20-dione	i) *Eubacterium desmolans* ii) *Clostridium cadavaris*	11β-Hydroxyandrost-4-ene-3,17-dione	[101]
11β,17α,21-Trihydroxy-pregn-4-ene-3,20-dione	*Eubacterium desmolans*	i) 3α,11β-Dihydroxy-5β-androstan-17-one ii) 3β,11β-Dihydroxy-5α-androstan-17-one iii) 11β-Hydroxy-5α-androstane-3,17-dione iv) 5β-Androstane-3α,11β,17β-triol	[101]
Pregna-4,9(11)-diene-3,20-dione	*Rhizopus stolonifer* ATCC 6227 b	Androsta-1,4,9(11)-triene-3,17-dione	[48]
3β-Hydroxypregn-5-en-20-one	*Coryneform bacteria* L 163 M	17β-Hydroxy-5α-androstan-3-one	[102]
3β-Hydroxypregna-5,16-dien-20-one	*Arthrobacter simplex* (IICB-321)	16α-Methoxy-17β-hydroxyandrosta-1,4-dien-3-one	[21,34]
17α-Hydroxypregn-4-ene-3,20-dione	*Clostridium scindens*	Androst-4-ene-3,17-dione	[103]
17α-Hydroxypregn-4-en-3,20-dione	*Arthrobacter simplex* (IICB-321)	i) Androst-4-ene-3,17-dione ii) Androsta-1,4-diene-3,17-dione	[21]
17α-Hydroxypregn-4-ene-3,20-dione	*Bacillus* sp.	Androst-4-ene-3,17-dione	[41]
17α-Hydroxypregn-4-ene-3,11,20-trione	*Clostridium scindens*	Antrost-4-ene-3,11,17-trione	[103]
17α,21-Dihydroxypregn-4-ene-3,11,20-trione	*Clostridium scindens*	Androst-4-ene-3,11,17-trione	[103]
17α,21-Dihydroxypregn-4-ene-3,20-dione	*Clostridium scindens*	Androst-4-ene-3,17-dione	[103]
17α,21-Dihydroxypregn-4-ene-3,20-dione	*Arthrobacter simplex* (IICB-321)	i) Androst-4-ene-3,17-dione ii) Androsta-1,4-diene-3,17-dione iii) 3aα-H-4α-(3′-propionic acid)-5α-hydroxy-7aβ-methylhexahydro-1-indanone-δ-lactone	[21]
11β,17α,21-Trihydroxypregn-4-ene-3,20-dione	*Clostridium* sp.	11β-Hydroxyandrost-4-ene-3,17-dione	[104]
11β,17α,21-Trihydroxypregn-4-ene-3,20-dione	*Clostridium scindens*	11β-Hydroxyandrost-4-ene-3,17-dione	[103]
3α,11β,17α,21-Tetrahydroxy-5β-pregnan-20-one	*Clostridium scindens*	3α,11β-Dihydroxy-5β-androstan-17-one	[103]
Sitosterol	*Mycobacterium* NRRL B-3805	Androst-4-ene-3,17-dione	[12]
Sitosterol	*Mycobacterium fortuitum* ATCC 6842 after mutation designated as NRRL B-15026	(7as)-2,3,7,7a-tetrahydro-7a-methyl-(1H)-indene-1,5-(6H)-dione	[35]
Sitosterol	*P. stutzeri*, *Micrococcus* sp. and *C. longisporum* (mixed culture)	i) Androst-4-ene-3,17-dione ii) 3β-Hydroxyandrost-5-en-17-one	[94]
Sitosterol	*Arthrobacter* sp. 317	i) Androsta-1,4-diene-3,17-dione ii) Androst-4-ene-3,17-dione	[95]
Sitosterol	*Rhodococcus australis* CSIR-236.457	3aα-H-4α-(3′-propionic acid)-5α-hydroxy-7aβ-methylhydro indan-1-one-δ-lactone	[20]
Sitosterol	*Mycobacterium* CCM 3528	22-Hydroxy-23,24-bisnor-chola-1,4-dien-3-one	[15]
Sitosterol	*Mycobacterium* CCM 3529	Androsta-1,4-diene-3,17-dione	[15]

Sitosterol	i) *Mycobacterium mucosum* 3-AP ii) *Mycobacterium flavum* B_2	Androsta-1,4-diene-3,17-dione	[14]
Sitosterol	i) *Mycobacterium mucosum* 3-AP ii) *Mycobacterium flavum* B_2	23,24-Bisnor-chola-1,4-dien-3-on-22-oic acid	[14]
Sitosterol	*Pseudomonas* NCIB 10590	i) Androsta-1,4-diene-3,17-dione ii) 3-Oxochol-4-en-24-oic acid iii) 3-Oxopregn-4-ene-20-carboxylic acid iv) Androst-4-ene-3,17-dione v) 3-Oxochola-1,4-dien-24-oic acid vi) Pregna-1,4-dien-3-one-20-carboxylic acid vii) 9α-Hydroxyandrosta-1,4-diene-3,17-dione	[58]
Sitosterol	*Rhodococcus* MIL 1037	i) Androst-4-en-3-one ii) Androsta-1,4-dien-3-one iii) Pregn-4-en-3-one-20-carboxylic acid iv) Pregna-1,4-dien-3-one-20-carboxylic acid	[105]
Sitosterol	*Mycobacterium fortuitum* ATCC 6842 mutant (UC-9778)	Methyl-3-hydroxy-9-oxo-9,10-seco-23,24-dinor-1,3,5(10)-cholatrienoate	[37]
Sitosterol	*Rhodococcus australis*	13aα-H-4α-(3′-propionic acid)-5α-hydroxy-7aβ-methylhexahydro-1-indanone-δ-lactone	[98]
Sitosterol	Mutant strain T-21	i) 9-Hydroxypregna-4,17(20)-dien-3-one-20-carbox aldehyde ii) 9,22-Dihydroxy-23,24-dinorchol-4-en-3-one	[38]
Sitosterol	*Mycobacterium fortuitum* N 10	9α-Hydroxyandrost-4-ene-3,17-dione	[59]
3β-Acetoxysitost-5-en-19-ol	*Moraxella* sp (immobilized)	Estrone	[94]
Stigmasterol	*Arthrobacter* sp. 317	i) Androst-4-ene-3,17-dione ii) Androsta-1,4-diene-3,17-dione	[95]
Stigmasterol	*Rhodococcus australis* CSIR 236.457	3aα-H-4α-(3′propionic acid)-5α-hydroxy-7aβ-methylhydroindan-1-one-δ-lactone	[20]
Stigmasterol	*Mycobacterium* CCM 3528	22-Hydroxy-23,24-bisnorchola-1,4-dien-3-one	[15]
Stigmasterol	*Mycobacterium* CCM 3529	Androsta-1,4-diene-3,17-dione	[15]
Stigmasterol	*Rhodococcus australis*	3aα-H-4α-(3′-propionic acid)-5α-hydroxy-7aβ-methylhexahydro-1-indanone-δ-lactone	[98]
3β-Methoxystigmast-5-ene	*Moraxella* sp.	3β-Methoxyandrost-5-en-17-one	[94]
Campesterol, sitosterol and stigmasterol mixture	*Arthrobacter* 317	i) Androst-4-ene-3,17-dione ii) Androsta-1,4-diene-3,17-dione	[106]
Sugarcane-sterols	*Arthrobacter globi-formis* strain oxydans	Androsta-1,4-diene-3,17-dione	[107]

Table 3. Dehydrogenation

Substrate	Microorganism	Product	Reference
5β-Androstrane-3,17-dione	*Clostridia* sp.	Androst-4-ene-3,17-dione	[108]
Androst-4-ene-3,17-dione	*Aphanocapsa* sp. (A. J. Smith 6308)	Androsta-1,4-diene-3,17-dione	[52]
3β-Hydroxyandrost-5-en-17-one	*Fusarium oxysporum* immobilized or free cell	Androsta-1,4-diene-3,17-dione	[109]
3β-Hydroxyandrost-5-en-17-one	*Pseudomonas testosteron*	i) Androst-4-ene-3,17-dione ii) 17β-Hydroxyandrost-4-en-3-one	[110]
11β-Hydroxyandrost-4-ene-3,17-dione	*Arthrobacter simplex* ATCC-6946	11β-Hydroxyandrosta-1,4-diene-3,17-dione	[111]
17β-Hydroxyandrost-4-en-3-one	*Arthrobacter* sp. 317	i) Androst-4-ene-3,17-dione ii) Androsta-1,4-diene-3,17-dione	[95]
17β-Hydroxyandrosta-4,6-dien-3-one	*Mucor griseocyanus* ATCC 1207	Androsta-4,6-diene-3,17-dione	[46]
17β-Hydroxy-5β-androstan-3-one	*Clostridia* sp.	17β-Hydroxyandrost-4-en-3-one	[108]
7β-Hydroxyandrost-4-en-3-one	*Corynebacterium* spp.	i) 3α-Hydroxy-5α-androstan-17-one ii) 3α-Hydroxy-5β-androstan-17-one	[112]
3β-Acetoxyandrost-5-en-17-one	*Flavobacterium dehydrogenans*	Androst-4-ene-3,17-dione	[31]
17β-Hydroxyandrost-4-en-3-one	*Corynebacterium* spp.	i) Androst-4-ene-3,17-dione ii) 5β-Androstane-3,17-dione iii) 3β-Hydroxy-5β-androstan-17-one	[112]
17β-Hydroxyandrost-4-en-3-one	i) *Aphanocapsa* sp. (A. J. Smith 6308) ii) *Characium californicum* (UTEX 2097)	Androst-4-ene-3,17-dione	[52]
17β-Hydroxyandrost-4-en-3-one	Mixed bacteria	i) Androst-4-ene-3,17-dione ii) 5β-Androstane-3,17-dione	[113]
17β-Hydroxy-A-nor-5α-androstan-2-one	*Cunninghamella elegans*	12β-Hydroxy-A-nor-5α-androstane-2,17-dione	[42]
3β,17β-Dihydroxyandrosta-4,6-diene	*Rhizopus arrhizus* ATCC-11145	6β,7β-Epoxy-17β-hydroxyandrost-4-en-3-one	[46]
Androsta-4,9(11)-diene-3,17-dione	*Arthrobacter simplex*	Androsta-1,4,9(11)-triene-3,17-dione	[28]
5β-Cholan-3-on-24-oic acid	*Clostridia* sp.	3-Oxochol-4-en-24-oic acid	[108]
3,12-Dioxo-5β-cholanoic acid	*Clostridia* sp.	3,12-Dioxochol-4-en-24-oic acid	[108]
3α-Hydroxy-5β-cholanoic acid	*Pseudomonas* NCIB 10590	i) Androsta-1,4-diene-3,17-dione ii) 3-Oxochol-4-en-24-oic acid iii) 17β-Hydroxyandrost-4-en-3-one iv) 17β-Hydroxyandrosta-1,4-dien-3-one v) 3-Oxo-5β-chol-24-oic acid vi) 3-Oxochola-1,4-dien-24-oic acid vii) 3-Oxopregn-4-ene-20-carboxylic acid viii) 3-Oxopregna-1,4-diene-20-carboxylic acid	[86]

6α-Hydroxy-3-oxo-5β-cholanic acid	*Clostridia* sp.	6α-Hydroxy-3-oxochol-4-en-24-oic acid	[108]
7α-Hydroxy-3-oxo-5β-cholanic acid	*Clostridia* sp.	7α-Hydroxy-3-oxochol-4-en-24-oic acid	[108]
12α-Hydroxy-3-oxo-5β-cholanic acid	*Clostridia* sp.	12α-Hydroxy-3-oxochol-4-en-24-oic acid	[108]
3α,7α-Dihydroxy-5β-cholanoic acid	*Pseudomonas* SD-102	3α-Hydroxy-7-oxo-5β-cholanic acid	[114]
3α,7α-Dihydroxy-5β-cholanoic acid	*Clostridium limosum* isolate F-14	3α-Hydroxy-7-oxo-5β-cholanoic acid	[115]
3α,7α-Dihydroxy-5β-cholanoic acid	*Clostridium absonum* (immobilized)	7-Oxo-3α-hydroxy-5β-cholanoic acid	[116]
3α,7β-Dihydroxy-5β-cholanoic acid	*Clostridium scindens*	3α-Hydroxy-7-oxo-5β-cholanoic acid	[103]
3α,11β-Dihydroxy-5β-cholanoic acid	*Alcaligenes faecalis* FERM BP-204	11β-Hydroxypregn-4-en-3-one-20-carbaldehyde	[17]
3α,12α-Dihydroxy-5β-cholanoic acid	*Pseudomonas putida* D 4014–A 1615	12β-Hydroxyandrost-4-ene-3,17-dione	[89]
3α,12α-Dihydroxy-5β-cholanoic acid	*Alcaligenes faecalis* D 4020–K 15	12α-Hydroxypregna-1,4-dien-3-one-20-carbaldehyde	[16]
3α,12α-Dihydroxy-5β-cholanoic acid	*Pseudomonas putida*	12α-Hydroxyandrosta-1,4-diene-3,17-dione	[91]
3α,12α-Dihydroxy-5β-cholanoic acid	*Alcaligenes faecalis*	12α-Hydroxypregna-1,4-dien-3-one-20-carboxylic acid	[92]
3α,7α,12α-Trihydroxy-5β-cholanoic acid	*Bacteroides fragilis* and *B. unoformis* (mixed culture)	3α,12α-Dihydroxy-7-oxo-5β-cholanoic acid	[117]
3α,7α,12α-Trihydroxy-5β-cholanoic acid-Na salt	*Pseudomonas putida* ATCC-31752	7α,12α-Dihydroxyandrosta-1,4-diene-3,17-dione	[29]
3α,7α,12α-Trihydroxy-5β-cholanoic acid	*Micrococcus* SD-101	3α,7α-Dihydroxy-12-oxo-5β-cholanoic acid	[118]
3α,7α,12α-Trihydroxy-5β-cholanoic acid	*Clostridium limosum isolate F*-14	3α,12α-Dihydroxy-7-oxo-5β-cholanoic acid	[115]
3α,7α,12α-Trihydroxy-5β-cholanoic acid	*Corynebacterium* SD-103	3α,7α-Dihydroxy-12-oxo-5β-cholanoic acid	[119]
7α,12α-Dihydroxy-3-oxo-5β-cholanoic acid	*Clostridia* sp.	7α,12α-Dihydroxy-3-oxochol-4-en-24-oic acid	[108]
5β-Cholanoic acid-3α-sulphate	*Pseudomonas* NCIB 10590	i) Androsta-1,4-diene-3,17-dione ii) 3-Oxopregna-1,4-diene-20-carboxylic acid	[87]
3α-Hydroxy-5β-cholanoic acid	*Corynebacterium* 320-6-2-1 FERM P-7521	i) 3-Oxopregna-1,4-diene-20-carboxylic acid ii) 3-Oxopregna-1,4,17(20)-triene-20-carboxylic acid iii) Androsta-1,4-diene-3,17-dione	[90]
		i) 12α-Hydroxyandrosta-1,4-diene-3,17-dione ii) 12α-Hydroxy-3-oxo-23,24-dinorchola-1,4-dien-22-oic acid	[39]
3α,12α-Dihydroxy-5β-cholanoic acid	*Pseudomonas* sp. mutant RAL 8.14	12β-Hydroxyandrosta-1,4-diene-3,17-dione	[39]
3α,12α-Dihydroxy-5β-cholanoic acid	*Pseudomonas* sp. mutant RAL 91.11	12α-Hydroxyandrosta-1,4-diene-3,17-dione	[39]
3α,12α-Dihydroxy-5β-cholanoic acid	i) *Pseudomonas* sp. mutant RAL 8.23 ii) *Pseudomonas* sp. mutant RAL 8.24 iii) *Pseudomonas* sp. mutant RAL 20.5		

Table 3. (*contd*)

Substrate	Microorganism	Product	Reference
3α,12α-Dihydroxy-5β-cholanoic acid	i) *Pseudomonas* sp. mutant RAL 1.22 ii) *Pseudomonas* sp. mutant RAL 8.15	12β-Hydroxyandrosta-1,4-diene-3,17-dione	[39]
Cholesterol	i) *Cephalosporium longisporum* ii) *Micrococcus* sp. iii) *Pseudomonas stutzeri*	i) Androst-4-ene-3,17-dione ii) Cholest-4-en-3-one	[94]
Cholesterol	*Pseudomonas stutzeri*, *Micrococcus* sp. and *Cephalosporium longisporum* (mixed culture)	i) Androst-4-ene-3,17-dione ii) Cholest-4-en-3-one	[94]
Cholesterol	*Moraxella* sp.	i) Cholest-4-en-3-one ii) Androst-4-ene-3,17-dione iii) Androsta-1,4-diene-3,17-dione	[94]
Cholesterol	*Arthrobacter* sp. 317	i) Androsta-1,4-diene-3,17-dione ii) Androst-4-ene-3,17-dione	[95]
Cholesterol	*Mycobacterium* CCM 3528	22-Hydroxy-23,24-bisnorchola-1,4-dien-3-one	[15]
Cholesterol	*Mycobacterium* CCM 3529	Androsta-1,4-diene-3,17-dione	[15]
Cholesterol	*Corynebacterium* 320-6-2-2	i) Pregn-4-en-3-one-20-carboxylic acid ii) Pregna-1,4-diene-3-one-20-carboxylic acid iii) Androsta-1,4-diene-3,17-dione	[13]
Cholesterol	*Mycobacterium* NRRL B-3805	17β-Hydroxyandrost-4-en-3-one	[18]
Cholesterol	*Mycobacterium phlei* (MYC-4)	i) 17β-Hydroxyandrost-4-en-3-one ii) 17β-Hydroxyandrosta-1,4-dien-3-one	[18]
Cholesterol	i) *Botrytis cinerea* strain 92 ii) *Sclerotinia* sp.	Cholest-4-en-3-one	[120]
Cholesterol	*Mycobacterium* CCM 3529	i) Androsta-1,4-diene-3,17-dione ii) Methyl-3-oxo-23,24-bisnor-1,4-choladienoate iii) Androst-4-ene-3,17-dione iv) 22-Hydroxy-23,24-bisnorchola-1,4-dien-3-one v) 17β-Hydroxyandrost-4-en-3-one vi) 17β-Hydroxyandrosta-1,4-dien-3-one	[96]
Cholesterol	*Pseudomonas* NCIB 10590	i) Cholest-4-en-3-one ii) Cholesta-1,4-dien-3-one iii) Androsta-1,4-diene-3,17-dione	[97]
Cholesterol acetate	*Moraxella* sp.	i) Cholest-4-en-3-one ii) Androsta-1,4-diene-3,17-dione	[94]

		iii) Androst-4-en-3,17-dione	
Cholest-5-en-3β-olhydrogen succinate	*Arthrobacter* sp. 317	i) Androsta-1,4-diene-3,17-dione ii) Androst-4-ene-3,17-dione	[95]
Diosgenin	*Nocardia rhodochrous*	Diosgenone	[121]
Pregn-4-ene-3,20-dione	*Arthrobacter simplex* (IICB-321)	i) Pregna-1,4-diene-3,20-dione ii) 17β-Hydroxyandrosta-1,4-dien-3-one iii) Androsta-1,4-diene-3,17-dione	[21]
Pregn-4-ene-3,20-dione	*Trichurus spiralis*	Androsta-1,4-diene-3,17-dione	[100]
Pregn-4-ene-3,20-dione	*Arthrobacter* sp. 317	Androsta-1,4-diene-3,17-dione	[95]
Pregn-4-ene-3,20-dione	*Colletotrichum antirrhini* SC-2144	i) Androsta-1,4-diene-3,17-dione ii) 14α-Hydroxyandrosta-1,4-diene-3,17-dione iii) 14α-Hydroxypregna-1,4-diene-3,20-dione	[66]
Pregna-4,8(9)-diene-3,20-dione	*Rhizopus stolonifer* ATCC 6227 b	9α,11α-Epoxyandrosta-1,4-diene-3,17-dione	[48]
Pregna-4,9(11)-diene-3,20-dione	*Rhizopus stolonifer* ATCC 6227 b	Androsta-1,4,9(11)-triene-3,17-dione	[48]
3β-Hydroxypregn-5-en-20-one	*Coryneform* bacteria L 163 M	17β-Hydroxy-5α-androstan-3-one	[102]
3β-Hydroxypregna-5,16-dien-20-one	*Arthrobacter simplex* (11CB-321)	i) Pregna-1,4,16-triene-3,20-dione ii) 16α-Methoxy-17β-hydroxyandrosta-1,4-dien-3-one	[21, 34]
17α-Hydroxypregn-4-ene-3,20-dione	*Arthrobacter simplex* (IICB-321)	Androsta-1,4-diene-3,17-dione	[21]
21-Acetoxy-17α-hydroxypregn-4-ene-3,11,20-trione	*Arthrobacter simplex* BY-2-13 (immobilized)	21-Acetoxy-17α-hydroxypregna-1,4-diene-3,11,20-trione	[122]
21-Acetoxy-17α-hydroxypregn-4-ene-3,11,20-trione	*Arthrobacter simplex*	21-Acetoxy-17α-hydroxypregna-1,4-diene-3,11,20-trione	[123]
3β,21-Diacetoxy-17α-hydroxy-16α-methyl-5α-pregn-9(11)-en-20-one	*Arthrobacter* 9-2	i) 21-Acetoxy-17α-hydroxy-16α-methylpregna-1,4,9(11)-triene-3,20-dione ii) 21-Acetoxy-9α,11α-epoxy-17α-hydroxy-16α-methylpregna-1,4-diene-3,20-dione	[124]
17α,21-Dihydroxypregn-4-ene-3,20-dione	*Arthrobacter simplex* (IICB-321)	i) Androsta-1,4-diene-3,17-dione ii) 17α,20β,21-Trihydroxy pregna-1,4-diene-3-one	[21]
17α,21-Dihydroxypregn-4-ene-3,20-dione	*Curvularia lunata* and *Arthrobacter simplex* (immobilized)	11β,17α,21-Trihydroxypregna-1,4-diene-3,20-dione	[23]
21-Acetoxy-3β,17α-dihydroxy-16β-methyl-5α-pregn-9(11)-en-20-one	*Arthrobacter* 9-2	9α,11α-Epoxy-17α,21-dihydroxy-16β-methyl-pregna-1,4-diene-3,20-dione	[125]
11β,17α,21-Trihydroxypregn-4-ene-3,20-dione	*Arthrobacter simplex* (immobilized)	11β,17α,21-Trihydroxypregna-1,4-diene-3,20-dione	[126, 129, 130, 131, 134, 24, 25]
11β,17α-21-Trihydroxypregn 4-ene-3,20-dione	*Arthrobacter globiformis* 193 (immobilized or free cell)	11β,17α,21-Trihydroxypregna-1,4-diene-3,20-dione	[127]
11β,17α,21-Trihydroxypregn-4-ene-3,20-dione	*Arthrobacter simplex*	11β,17α,21-Trihydroxypregna-1,4-diene-3,20-dione	[30, 128, 135]

Table 3. (*contd*)

Substrate	Microorganism	Product	Reference
11β,17α,21-Trihydroxypregn-4-ene-3-20-dione	*Arthrobacter simplex* BY-2-13 (immobilized)	11β,17α,21-Trihydroxypregna-1,4-diene-3,20-dione	[122]
11β,17α,21-Trihydroxypregn-4-ene-3,20-dione	*Corynebacterium simplex* (immobilized)	11β,17α,21-Trihydroxypregna-1,4-diene-3,20-dione	[132]
11β,17α,21-Trihydroxypregn-4-ene-3,20-dione	*Arthrobacter globiformis* 193	i) 11β,17α,21-Trihydroxypregna-1,4- diene-3,20-dione ii) 11β,17α,20β,21-Tetrahydroxypregna-1,4-dien-3-one	[133]
11β,17α,21-Trihydroxypregn-4-ene-3,20-dione	*Arthrobacter globiformis*	11β,17α,21-Trihydroxypregna-1,4-diene-3,20-dione	[136]
11β,17α,21-Trihydroxypregn-4-ene-3,20-dione	*Arthrobacter simplex*	11β,17α,21-Trihydroxypregna-1,4-diene-3,20-dione	[137]
17α,21-Dihydroxypregn-4-ene-3,11,20-trione	*Mycobacterium flavum*	17α,21-Dihydroxypregna-1,4-diene-3,11,20-trione	[138]
3β,21-Diacetoxy-17α-hydroxy-16α-methyl-5α-pregn-9(11)-en-20-one	*Mycobacterium smegmatis* and *Arthrobacter simplex* (mixed culture)	i) 17α,21-Dihydroxy-16α-methylpregna-1,4,9(11)-triene-3,20-dione ii) 17α,21-Dihydroxy-9α,11α-epoxy-16α-methyl-pregna-1,4-diene-3,20-dione	[139]
11β,17α,21-Trihydroxypregn-4-ene-3,20-dione	*Arthrobacter simplex* BY-2-13	11β,17α,21-Trihydroxypregna-1,4-diene-3,20-dione	[140]
3β,17α,21-Trihydroxy-5α-pregnan-20-one	*Arthrobacter* 9-2	17α,20,21-Trihydroxypregna-1,4-dien-3-one	[141]
3β-Acetoxy-17α-hydroxy-16α-methyl-5α-pregnan-20-one	*Mycobacterium smegmatis* and *Arthrobacter simplex* (mixed culture)	17α,Hydroxy-16α-methylpregna-1,4-diene-3,20-dione	[72]
Sitosterol	*Mycobacterium* NRRL B-3805	Androst-4-enc-3,17-dione	[12]
Sitosterol	*Pseudomonas stutzeri*, *Micrococcus* sp. and *Cephalosporium longisporum* (mixed culture)	Androst-4-ene-3,17-dione	[94]
Sitosterol	*Arthrobacter* sp. 317	i) Androsta-1,4-diene-3,17-dione ii) Androst-4-ene-3,17-dione	[95]
Sitosterol	*Mycobacterium* CCM 3528	22-Hydroxy-23,24-bisnorchola-1,4-dien-3-one	[15]
Sitosterol	*Mycobacterium* CCM 3529	Androsta-1,4-dien-3,17-dione	[15]
Sitosterol	i) *Mycobacterium mucosum* 3-AP ii) *Mycobacterium flavum* B_2	i) Androsta-1,4-diene-3,17-dione ii) 23,24-Bisnorchola-1,4-dien-3-on-22-oic acid	[14]
Sitosterol	*Pseudomonas* NCIB 10590	i) 24-Ethylcholest-4-en-3-one ii) Androsta-1,4-diene-3,17-dione iii) 3-Oxochol-4-en-24-oic acid iv) 3-Oxopregn-4-en-20-carboxylic acid v) 26-Hydroxy-24-ethylcholest-4-en-3-one vi) Androst-4-ene-3,17-dione vii) 3-Oxo-24-ethylcholest-4-en-26-oic acid viii) 3-Oxochola-1,4-dien-24-oic acid ix) Pregna-1,4-dien-3-one-20-carboxylic acid	[58]

		x) 9α-Hydroxyandrosta-1,4-diene-3,17-dione	
Sitosterol	*Rhodococcus* MIL 1037	i) Pregna-1,4-dien-3-one-20-carboxylic acid ii) Androsta-1,4-dien-3-one iii) Pregn-4-en-3-one-20-carboxylic acid iv) Androst-4-en-3-one	[105]
Sitosterol	Mutant strain T-21	i) 9-Hydroxypregna-4,17(20)-dien-3-one-20-carboxaldehyde ii) 9,22-Dihydroxy-23,24-dinor-chol-4-en-3-one	[38]
Sitosterol	*Mycobacterium fortuitum* N 10	9α-Hydroxyandrost-4-ene-3,17-dione	[59]
Stigmasterol	*Arthrobacter* sp. 317	i) Androsta-1,4-diene-3,17-dione ii) Androst-4-ene-3,17-dione	[95]
Stigmasterol	*Mycobacterium* CCM 3528	22-Hydroxy-23,24-bisnorchola-1,4-dien-3-one	[15]
Stigmasterol	*Mycobacterium* CCM 3529	Androsta-1,4-diene-3,17-dione	[15]
Sitosterol, stigmasterol and campesterol mixture	*Arthrobacter* 317	i) Androst-4-ene-3,17-dione ii) Androsta-1,4-diene-3,17-dione	[106]
Sugarcane-sterols	*Arthrobacter globiformis* strain oxydans	Androsta-1,4-diene-3,17-dione	[107]

Table 4. Reduction of ketro group

Substrate	Microorganism	Product	Reference
3C=0 → 3α-OH			
11β-Hydroxyandrost-4-ene-3,17-dione	*Bacteroides fragilis*	5β-Androstane-3α,11β,17β-triol	[142]
17β-Hydroxyandrost-4-en-3-one	*Corynebacterium* spp.	i) 3α-Hydroxy-5α-androstan-17-one ii) 3α-Hydroxy-5β-androstan-17-one iii) 5β-Androstane-3α,17α-diol	[112]
4-Choloro-11β,17β-dihydroxy-17α-methylandrost-4-en-3-one	*Clostridium paraputrificum* H-2	4-Chloro-17α-methyl-5β-androstane-3α,11β,17β-triol	[143]
11β,17α,21-Trihydroxypregn-4-ene-3,20-dione	*Eubacterium desmolans*	i) 3α-,11β-Dihydroxy-5β-androstan-17-one ii) 5β-Androstane-3α,11β,17β-triol	[101]
3-Oxo-5β-cholanoic acid	*Clostridium perfringens*	3α-Hydroxy-5β-cholanoic acid	[144]
3C=0 → 3β-OH			
D-Homo-5α-androstane-3,17a-dione	*Cunninghamella elegans*	1β,3β-Dihydroxy-D-homo-5α-androstane-7,17a-dione	[43]
17β-Hydroxyandrost-4-en-3-one	*Corynebacterium* spp.	3β-Hydroxy-5β-androstan-17-one	[112]
3-Oxo-5β-cholanoic acid	*Clostridium perfringens*	3β-Hydroxy-5β-cholanoic acid	[144]
Cholest-4-en-3-one	*Pseudomonas stutzeri*, *Micrococcus* sp. and *Cephalosporium longisporum* (mixed culture)	3β-Hydroxyandrost-5-en-17-one	[94]
4-Chloro-11β,17β-dihydroxy-17α-methylandrost-4-en-3-one	*Rhodotorula glutinis* H-86	4-Chloro-17α-methyl-5α-androst-3β,11β,17β-triol	[143]

Table 4. (*contd*)

Substrate	Microorganism	Product	Reference
$11\beta,17\alpha,21$-Trihydroxypregn-4-en-3,20-dione	*Eubacterium desmolans*	$3\beta,11\beta$-Dihydroxy-5α-androstan-17-one	[101]
17C=0→17β-OH			
Androst-4-ene-3,17-dione	*Bacteroides fragilis*	17β-Hydroxyandrost-4-en-3-one	[142]
Androst-4-ene-3,17-dione	i) *Anabaena flos-aquae* UTEX 1444 ii) *Anabaena cylindrica* (IVL) iii) *Anacystis nidulas* (SVAL 1402-1) iv) *Characium californicum* (UTEX 2097) v) *Chlorococcum macrostigmatum* (UTEX-109) vi) *Coccochloris peniocystis* (UTEX 1548) vii) *Coelastrum proboscideum* var. *grucile* (ATCC 30410) viii) *Oocystis* sp (ATCC-30417) ix) *Oscillatoria prolifera* UTEX 1270 x) *Phormidium autumnale* UTEX 1580 xi) *Scenedesmus quadricauda* ATCC 11460	17β-Hydroxyandrost-4-en-3-one	[52]
Androst-4-ene-3,17-dione	i) *Ankistrodesmus angustus* ATCC 30448 ii) *Scenedesmus quadricauda* ATCC 11460	$14\alpha,17\beta$-Dihydroxyandrost-4-en-3-one	[52]
Androsta-4,6-diene-3,17-dione	*R. arrhizus* ATCC-11145	i) 17β-Hydroxyandrosta-4,6-dien-3-one ii) 17β-Hydroxyandrosta-4,6-diene-3,16-dione	[46]
3β-Acetoxyandrost-5-en-17-one	*Streptomyces albus*	17β-Hydroxyandrost-4-en-3-one	[145]
3β-Hydroxyandrost-5-en-17-one	*Pseudomonas testosteroni*	17β-Hydroxyandrost-4-en-3-one	[110]
11β-Hydroxyandrost-4-ene-3,17-dione	*Bacteroides fragilis*	i) 5β-Androstane-$3\alpha,11\beta,17\beta$-triol ii) $11\beta,17\beta$-Dihydroxyandrost-4-en-3-one	[142]
Estrone	i) *Clostridium paraputrificum* ii) *Clostridium* J-1 iii) *Clostridium innocuum* iv) *Eubacterium lentum*	Estra-1,3,5(10)-trien-3,17β-diol	[142]
3,16α-Dihydroxyestra-1,3,5(10)-trien-17-one	i) *Clostridium paraputrificum* ii) *Clostridium* J-1 iii) *Clostridium innocuum* iv) *Eubacterium lentum*	Estra-1,3,5(10)-triene-3,$16\alpha,17\beta$-triol	[142]

Table 4. (*contd*)

Substrate	Microorganism	Product	Reference
3-Methoxy-8,14-seco-1,3,5(10),9(11)-estratetraene-14,17-dione	*Pichia farinosa* Y-118	3-Methoxy-17β-hydroxy-8,14-seco-1,3,5(10),9(11)-estrtetraen-14-one	[146]
3-Methoxy-8,14-seco-1,3,5(10),9(11)-estratetraene-14,17-dione	*Pichia farinosa* Y-118 (immobilized)	3-Methoxy-17β-hydroxy-8,14-seco-1,3,5(10),9(11)-estratetraen-14-one	[147]
<u>20C=0→20α-OH</u>			
3β-Acetoxypregn-5-en-20-one	*Streptomyces albus*	20α-Hydroxypregn-4-en-3-one	[145]
17α-Hydroxypregn-4-ene-3,20-dione	*Clostridium scindens*	17α,20α-Dihydroxypregn-4-en-3-one	[103]
17α-Hydroxypregn-4-ene-3,20-dione	*Bacillus* sp.	6β,17α,20α-Trihydroxypregn-4-en-3-one	[41]
17α-Hydroxypregn-4-ene-3,11,20-trione	*Clostridium scindens*	17α,20α-Dihydroxypregn-4-ene-3,11-dione	[103]
21-Hydroxypregn-4-ene-3,20-dione	*Clostridium scindens*	20α,21-Dihydroxypregn-4-en-3-one	[103]
21-Hydroxypregn-4-ene-3,11,20-trione	*Clostridium scindens*	20α,21-Dihydroxypregn-4-ene-3,11-dione	[103]
11β,21-Dihydroxypregn-4-ene-3,20-dione	*Clostridium scindens*	11β,20α,21-Trihydroxypregn-4-en-3-one	[103]
17α,21-Dihydroxypregn-4-ene-3,20-dione	*Clostridium scindens*	17α,20α,21-Trihydroxypregn-4-en-3-one	[103]
17α,21-Dihydroxypregn-4-ene-3,11,20-trione	*Clostridium scindens*	17α,20α,21-Trihydroxypregn-4-ene-3,11-dione	[103]
11β,17α,21-Trihydroxypregn-4-ene-3,20-dione	*Clostridium scindens*	11β,17α,20α,21-Tetrahydroxypregn-4-en-3-one	[103]
3α,11β,17α,21-tetrahydroxy-5β-pregnan-20-one	*Clostridium scindens*	5β-Pregnane-3α,11β,17α,20α,21-pentol	[103]
<u>20C=0→20β-OH</u>			
17α,21-Dihydroxypregn-4-ene-3,20-dione	*Arthrobacter simplex* (IICB-321)	Pregna-1,4-dien-3-one-17,20β,21-triol	[21]
11β,17α,21-Trihydroxypregn-4-ene-3,20-dione	*Arthrobacter globiformis*	11β,17α,20β,21-Tetrahydroxypregn-4-en-3-one	[148]
11β,17α,21-Trihydroxypregn-4-ene-3,20-dione	*Arthrobacter globiformis* 193	i) 11β,17α,20β,21-Tetrahydroxypregn-4-en-3-one ii) 11β,17α,20β,21-Tetrahydroxypregna-1,4-dien-3-one	[133]
11β,17α,21-Trihydroxypregna-1,4-diene-3,20-dione	*Arthrobacter globiformis*	11β,17α,20β,21-Tetrahydroxypregna-1,4-diene-3-one	[148]
11β,17α,21-Trihydroxypregna-1,4-diene-3,20-dione	*Arthrobacter globiformis* 193	i) 11β,17α,20β,21-Tetrahydroxypregna-1,4-dien-3-one ii) 11β,17α,20β,21-Tetrahydroxypregn-4-en-3-one	[133]
11β,17α,21-Trihydroxypregn-4-ene-3,20-dione	i) *Eubacterium desmolans* ii) *Clostridium cadavaris*	11β,17α,20β,21-Tetrahydroxypregn-4-en-3-one	[101]
3β,17α,21-Trihydroxy-5α-pregnan-20-one	*Arthrobacter* 9-2	17α,20,21-Trihydroxypregna-1,4-dien-3-one	[141]

Table 5. Miscellaneous reaction

Substrate	Microorganism	Product	Reference
Aromatization			
Androst-4-ene-3,17-dione	Mutant strain of *Nocardia restricta*	3-Hydroxy-9,10-secoandrosta-1,3,5(10)- triene-9,17-dione	[149]
19-Nor-17β-hydroxyandrost-4-en-3-one	*Aspergillus ochraceus* ATCC-1008 and *Arthrobacter simplex* ATCC-6946 (mixed culture)	3,11α-Dihydroxyestra-1,3,5(10)-trien-17-one	[61]
3α,12α-Dihydroxy-5β-cholanoic acid	i) *Pseudomonas* sp. mutant RAL 1.25 ii) *Pseudomonas* sp. mutant RAL 8.16 iii) *Pseudomonas* sp. mutant RAL 20.8	3,12β-Dihydroxy-9(10)-secoandrosta-1,3,5(10)-triene-9,17-dione	[39]
3β-Acetoxycholest-5-en-19-ol	*Moraxella* sp. (immobilized)	Estrone	[94]
19-Hydroxycholesteryl acetate	*Moraxella* sp. (immobilized)	Estrone	[27]
Pregn-4-ene-3,20-dione	*Arthrobacter simplex IICB*-321	3-Hydroxy-9,10-seco-1,3,5(10)-androstatriene-9,17-dione	[21]
3β-Acetoxysitost-5-en-19-ol	*Moraxella* sp. (immobilized)	Estrone	[94]
Dehydration			
3α,7α,12α-Trihydroxy-5β-cholanoic acid	*Bacteroides fragilis* and *Bacteroides uniformis* (mixed culture)	3α,12α-Dihydroxy-5α-chol-6-enoic acid	[117]
Dehydroxylation			
3α,7α-Dihydroxy-5β-cholanoic acid	*Clostridium scindens*	3α-Hydroxy-5β-cholanoic acid	[103]
3α,7β-Dihydroxy-5β-cholanoic acid	*Eubacterium* strain C-25 and *Bacteroides distasonis* strain K-5	3α-Hydroxy-5β-cholanoic acid	[150]
3α,7α,12α-Trihydroxy-5β-cholanoic acid	*Clostridium scindens*	3α,12α-*Dihydroxy-5β-cholanoic acid*	[103]
3α,7α,12α-Trihydroxy-5β-cholanoic acid	*Bacteroides fragilis* and *Bacteroides uniformis* (mixed culture)	3α,12α-Dihydroxy-5α-cholanoic acid	[117]
3α,7α,12α-Trihydroxy-5β-cholanoic acid	i) *Bacteroides distasonis* ii) *Bacteroides eggerthii* iii) *Bacteroides fragilis* iv) *Bacteroides incommunis* v) *Bacteroides thetaiotaomicron* vi) *Bacteroides uniformis* vii) *Bacteroides variabilis* viii) *Bacteroides vultatus* ix) Eubacterium sp. x) *Lactobacillus* sp.	3α,12α-Dihydroxy-5β-chol-24-oic, methyl ester	[151]

5β-Pregnane-3α,21-diol-20-one	*Eubacterium lentum* ATCC-25559 and *Escherichia coli* (mixed culture)	5β-Pregnan-3α-ol-20-one	[152]
3α,21-Dihydroxy-5β-pregnan-20-one	*Eubacterium lentum*	3α-Hydroxy-5β-pregnan-20-one	[153]
<u>Epoxidation</u>			
5α-Androst-6-ene-3,17-dione	*Rhizopus arrhizus* ATCC-11145	6α,7α-Epoxy-5α-androstane-3,17-dione	[46]
5β-Androst-6-ene-3,17-dione	*Rhizopus arrhizus* ATCC-11145	6α,7α-Epoxy-5β-androstane-3,17-dione	[46]
Androsta-1,4-diene-3,17-dione	*Rhizopus arrhizus* ATCC-11145	1β,2β-Epoxyandrost-4-ene-3,17-dione	[45]
Androsta-4,6-diene-3,17-dione	*Rhizopus arrhizus* ATCC-11145	6β,7β-Epoxyandrost-4-ene-3,17-dione	[46]
Androsta-4,6-diene-3,17-dione	*Mucor griseocyanus* ATCC-1207	6α,7α-Epoxyandrost-4-ene-3,17-dione	[46]
17β-Hydroxyandrosta-4,6-dien-3-one	*Rhizopus arrhizus* ATCC-11145	6β,7β-Epoxy-17β-hydroxyandrost-4-en-3-one	[46]
3β,17β-Dihydroxyandrosta-4,6-diene	*Rhizopus arrhizus* ATCC-11145	6β,7β-Epoxy-17β-hydroxyandrost-4-en-3-one	[46]
17β-Hydroxyestra-4,6-dien-3-one	*Rhizopus arrhizus* ATCC-11145	6β,7β-Epoxy-17β-hydroxyestr-4-en-3-one	[46]
Pregna-4,8(9)-diene-3,20-dione	*Rhizopus stolonifer* ATCC-6227b	9α-11α-Epoxyandrosta-1,4-diene-3,17-dione	[48]
Pregna-4,8(9)-diene-3,20-dione	*Mucor griseocyanus* ATCC-1207	9α,11α-Epoxy-6β-hydroxypregn-4-ene-3,20-dione	[48]
Pregna-4,9(11)-diene-3,20-dione	*Curvularia lunata* NRRL-2380	9β,11β-Epoxypregn-4-ene-3,20-dione	[48]
Pregna-4,9(11)-diene-3,20-dione	*Mucor griseocyanus* ATCC-1207	9α,11α-Epoxypregn-4-ene-3,20-dione	[48]
21-Acetoxy-3β,17α-dihydroxy-16β-methyl-5α-pregn-9(11)-en-20-one	*Arthrobacter* 9-2	9α,11α-Epoxy-17α,21-dihydroxy-16β-methylpregna-1,4-diene-3,20-dione	[125]
3β,21-Diacetoxy-17α-hydroxy-16α-methyl-5α-pregn-9(11)-en-20-one	*Arthrobacter* 9-2	21-acetoxy-9α,11α-epoxy-17α-hydroxy-16α-methyl-Pregna-1,4-diene-3,20-dione	[124]
3β,21-Diacetoxy-17α-hydroxy-16α-methyl-5α-pregn-9(11)-en-20-one	*Mycobacterium smegmatis* and *Arthrobacter simplex* (mixed culture)	17α,21-Dihydroxy-9α,11α-epoxy-16α-methylpregna-1,4-diene-3,20-dione	[139]
<u>Esterification</u>			
3α,7α,12α-Trihydroxy-5β-cholanoic acid	i) *Bacteroides distasonis* ii) *Bacteroides eggerthii* iii) *Bacteroides fragilis* iv) *Bacteroides incommunis* v) *Bacteroides thetaiotaomicron* vi) *Bacteroides uniformis* vii) *Bacteroides variabilis* viii) *Bacteroides vultatus* ix) *Eubacterium* sp. x) *Lactobacillus* sp.	3α,12α-Dihydroxy-5β-chol-24-oic, methyl ester	[151]
Cholesterol	*Mycobacterium* CCM 3529	Methyl-3-oxo-23,24-bisnor-1,4-choladienoate	[96]
Cholesterol	*Mycobacterium fortuitum* ATCC-6842 mutant (UC-9778)	Methyl-3-hydroxy-9-oxo-9,10-*seco*-23,24-dinor-1,3,5(10)-cholatrienoate	[37]
Sitosterol	*Mycobacterium fortuitum*		
Sitosterol	*Mycobacterium fortuitum* ATCC-6842 mutant (UC-9778)	Methyl-3-hydroxy-9-oxo-9,10-*seco*-23,24-dinor-1,3,5(10)-cholatrienoate	[37]
<u>Hydrogenation</u>			
11β-Hydroxyandrost-4-ene-3,17-dione	*Bacteroides fragilis*	5β-Androstane-3α,11β,17β-triol	[142]
17β-Hydroxyandrost-4-en-3-one	*Coryneform* bacteria L 163 M	i) 17β-Hydroxy-5α-androstan-3-one ii) 17β-Hydroxy-5β-androstan-3-one	[102]

Table 5. (*contd*)

Substrate	Microorganism	Product	Reference
17β-Hydroxyandrost-4-en-3-one	*Coryneform* bacteria	i) 17β-Hydroxy-5α-androstan-3-one ii) 17β-Hydroxy-5β-androstan-3-one	[154]
17β-Hydroxyandrost-4-en-3-one	*Corynebacterium* spp.	i) 3α-Hydroxy-5β-androstan-17-one ii) 3α-Hydroxy-5α-androstan-17-one iii) 5β-Androstane-3,17-dione iv) 3β-Hydroxy-5β-androstan-17-one v) 5β-Androstane-3α,17α-diol	[112]
17β-Hydroxyandrost-4-en-3-one	Mixed bacteria	i) 17β-Hydroxy-5α-androstan-3-one ii) 5β-Androstane-3,17-dione iii) 17α-Hydroxy-5β-androstan-3-one	[113]
4-Chloro-11β,17β-dihydroxy-17α-methylandrost-4-en-3-one	*Clostridium paraputrificum* H-2	i) 4-Chloro-11β,17β-dihydroxy-17α-methyl-5β-androstan-3-one ii) 4-Chloro-17α-methyl-3α,11β,17β-trihydroxy-5β-androstane	[143]
4-Chloro-11β,17β-dihydroxy-17α-methylandrosta-		4-Chloro-11β,17β-dihydroxy-17α-methyl-5β-androst-1-en-3-one	[143]
1,4-dien-3-one	*Clostridium paraputrificum* H-2	4-Chloro-17α-methyl-5α-androst-3β,11β,17β-triol	[143]
4-Chloro-11β,17β-dihydroxy-17α-methylandrosta-4-	*Rhodotorula glutinis* H-86		
en-3-one		i) 5β-Androstane-3α,11β,17β-triol	[101]
11β,17α,21-Trihydroxypregn-4-ene-3,20-dione	*Eubacterium desmolans*	ii) 11β-Hydroxy-5α-androstane-3,17-dione iii) 3β,11β-Dihydroxy-5α-androstan-17-one	
		9α,14α-Dihydroxypregn-4-ene-3,20-dione	[48]
Pregna-4,8(9)-diene-3,20-dione	*Curvularia lunata* NRRL 2380	Pregn-5-ene-3,20-dione	[155]
Pregna-4,16-diene-3,20-dione	*Eubacterium* sp. 144	17β-Hydroxy-5α-androstan-3-one	[102]
3β-Hydroxypregn-5-en-20-one	*Coryneform* bacteria L 163 M	i) 11β,17α,21-Trihydroxypregn-4-ene-3,20-dione	[133]
11β,17α,21-Trihydroxypregna-1,4-diene-3,20-dione	*Arthrobacter globiformis* 193	ii) 11β,17α,20β,21-Tetrahydroxypregn-4-en-3-one	
Hydrolysis		3β-Hydroxyandrost-5-en-17-one	[145]
3β-Acetoxyandrost-5-en-17-one	*Streptomyces albus*	17β-Hydroxyandrost-4-en-3-one	[145]
17β-Acetoxyandrost-4-en-3-one	*Streptomyces albus*	3β,11α-Dihydroxy-D-homo-5α-androstan-17a-one	[43]
3β-Acetoxy-D-homo-5α-androstan-17a-one	*Aspergillus ochraceus*	i) 3β-Hydroxy-D-homo-5α-androstane-7,17a-dione	[43]
3β-Acetoxy-D-homo-5α-androstan-17a-one	*Cunninghamella elegans*	ii) 3β,7β-Dihydroxy-D-homo-5α-androstan-17a-one iii) 1β,3β-Dihydroxy-D-homo-5α-androstane-7,17a-dione iv) 1β,3β,7β-Trihydroxy-D-homo-5α-androstan-17a-one v) 1β,3β,7α-Trihydroxy-D-homo-5α-androstan-17a-one	
		3β,9α,14α-Trihydroxy-D-Trihydroxy-D-homo-5α-androstan-17-one	[43]
3β-Acetoxy-D-homo-5α-androstan-17-one	*Cunninghamella elegans*	3α-Hydroxy-5β-cholanoic acid	[87]
5β-Cholanoic acid-3α-sulphate	*Pseudomonas* NCIB 10590	Cholesterol	[156]
Cholesterol acetate	*Bacillus* sp.	Cholesterol	[157]
β-D-Cholesteryl glucoside	*Nocardia restricta* CBS 157.45		

3β-Acetoxycholest-5-en-19-ol	*Moraxella* sp. (immobilized)	Estrone	[94]
6β-Methoxy-3α,5α-cyclocholestane	*Moraxella* sp.	*i*-Cholesterol	[94]
19-Hydroxycholesteryl acetate	*Moraxella* sp. (immobilized)	Estrone	[27]
3β-Acetoxypregn-5-en-20-one	*Streptomyces albus*	3β-Hydroxypregn-5-en-20-one	[145]
3β-Acetoxypregna-5,16-dien-20-one	*Streptomyces albus*	3β-Hydroxypregna-5,16-dien-20-one	[145]
21-Acetoxy-17α-hydroxypregn-4-ene-3,20-dione	*Absidia orchidis*	11β,17α,21-Trihydroxypregn-4-ene-3,20-dione	[76]
21-Acetoxy-17α-hydroxypregn-4-ene-3,20-dione	i) *Curvularia geniculata* ii) *Curvularia lunata* iii) *Curvularia inaequalis*	i) 6β,17α,21-Trihydroxypregn-4-ene-3,20-dione ii) 11α,17α,21-Trihydroxypregn-4-ene-3,20-dione iii) 11β,17α,21-Trihydroxypregn-4-ene-3,20-dione iv) 14α,17α,21-Trihydroxypregn-4-ene-3,20-dione	[49]
21-Acetoxy-17α-hydroxypregn-4-ene-3,20-dione	*Curvularia lunata* BKM F-644	11β,17α,21-Trihydroxypregn-4-ene-3,20-dione	[78]
21-acetoxy-17α-hydroxy-pregn-4-ene-3,11,20-trione	*Arthrobacter citreus* VKM 654 (immobilized or free cell)	17α,21-Dihydroxypregn-4-ene-3,11,20-trione	[158]
21-Acetoxy-17α-hydroxypregna-1,4-diene-3,11,20-trione	*Arthrobacter citreus* VKM 654 (immobilized or free cell)	17α,21-Dihydroxypregna-1,4-diene-3,11,20-trione	[158]
21-Acetoxy-3β,17α-dihydroxy-16β-methyl-5α-pregn-9(11)-en-20-one	*Arthrobacter* 9-2	9α,11α-Epoxy-17α,21-dihydroxy-16β-methylpregna-1,4-diene-3,20-dione	[125]
21-Acetoxy-11α,17α-dihydroxypregn-4-ene-3,20-dione	*Arthrobacter citreus* VKM 654 (immoblized or free cell)	11α,17α,21-Trihydroxypregn-4-ene-3,20-dione	[158]
21-Acetoxy-11α,17α-dihydroxypregna-1,4-diene-3,20-dione	*Arthrobacter citreus* VKM 654 (immobilized or free cell)	11α,17α,21-Trihydroxypregna-1,4-diene-3,20-dione	[158]
3β-Acetoxysitost-5-en-19-ol	*Moraxella* sp. (immobilized)	Estrone	[94]
21-Acetoxy-17α-hydroxy-16α-methylpregn-4-ene-3,20-dione	*Beauveria bassiana* AS 69	16α-Methyl-11α,17α,21-Trihydroxypregn-4-ene-3,20-dione	[73]
3β,21-Diacetoxy-17α-hydroxy-16α-methyl-5α-pregn-9(11)-en-20-one	*Mycobacterium smegmatis* and *Arthrobacter simplex* (mixed culture)	i) 17α,21-Dihydroxy-16α-methylpregna-1,4,9(11)-triene-3,20-dione ii) 17α,21-Dihydroxy-9α,11α-epoxy-16α-methylpregna-1,4-diene-3,20-dione	[139]
Hydrolysis and oxidation			
3β-Acetoxyandrost-5-en-17-one	*Streptomyces albus*	i) Androst-4-ene-3,17-dione ii) 17β-Hydroxyandrost-4-en-3-one	[145]
17β-Acetoxyandrost-4-en-3-one	*Streptomyces albus*	Androst-4-ene-3,17-dione	[145]
5β-Cholanoic acid-3α-sulphate	*Pseudomonas* NCIB 10590	i) Androsta-1,4-diene-3,17-dione ii) 3-Oxopregna-1,4-diene-20-carboxylic acid	[87]
Cholesteryl acetate	*Moraxella* sp.	i) Androst-4-ene-3,17-dione ii) Androsta-1,4-diene-3,17-dione iii) Cholest-4-en-3-one	[94]
3β-Acetoxypregn-5-en-20-one	*Streptomyces albus*	i) Pregn-4-ene-3,20-dione ii) 20α-Hydroxypregn-4-en-3-one	[145]
3β-Acetoxypregna-5,16-dien-20-one	*Streptomyces albus*	Pregna-4,16-diene-3,20-dione	[145]
3β,21-Diacetoxy-17α-hydroxy-16α-methyl-5α-pregn-9(11)-en-20-one	*Mycobacterium smegmatis* and *Arthrobacter simplex* (mixed culture)	i) 17α,21-Dihydroxy-16α-methylpregna-1,4,9(11)-triene-3,20-dione ii) 17α,21-Dihydroxy-9α,11α-epoxy-16α-methylpregna-1,4-diene-3,20-dione	[139]

Table 5. (*contd*)

Substrate	Microorganism	Product	Reference
3β-Acetoxyandrost-5-en-17-one	*Flavobacterium dehydrogenans*	Androst-4-ene-3,17-dione	[31]
3β-Acetoxy-17α-hydroxy-16α-methyl-5α-pregnan-20-one	*Mycobacterium smegmatis* and *Arthrobacter simplex* (mixed culture)	17α-Hydroxy-16α-methylpregna-1,4-diene-3,20-dione	[72]
3β,21-Diacetoxy-17α-hydroxy-16α-methyl-5α-pregn-9(11)-en-20-one	*Arthrobacter* 9-2	i) 21-Acetoxy-17α-hydroxy-16-methylpregna-1,4,9(11)-triene-3,20-dione ii) 21-Acetoxy-9α,11α-epoxy-17α-hydroxy-16α-methylpregna-1,4-diene-3,20-dione	[124]
Proscillaridin A	*Nocardia restricta* CBS 157.45	3-Keto-14β-hydroxybufa-1,4,20,22-tetraenolide	[157]
Isomerization			
4-Choloro-11β,17β-dihydroxy-17α-methylandrost-4-en-3-one	*Rhodotorula glutinis* H-86	4-Chloro-17α-methyl-5α-androst-3β,11β,17β-triol	[143]
3α,12α-Dihydroxy-5β-cholanoic acid	i) *Pseudomonas* sp. mutant RAL 1.22 ii) *Pseudomonas* sp. mutant RAL 8.15 iii) *Pseudomonas* sp. mutant RAL 91.11	12β-Hydroxyandrosta-1,4-diene-3,17-dione	[39]
3α,12α-Dihydroxy-5β-cholanoic acid	i) *Pseudomonas* sp. mutant RAL 1.25 ii) *Pseudomonas* sp. mutant RAL 8.16 iii) *Pseudomonas* sp. mutant RAL 20.8	3,12β-Dihydroxy-9(10)-secoandrosta-1,3,5(10)-triene-9,17-dione	[39]
3α,7α-Dihydroxy-5β-cholanoic acid	*Pseudomonas* sp. mutant RAL 8.23	3,7-Dihydroxy-4(5),(9(10)-diseco-5,9,17-trioxoandrosta-1(10),2-dien-4-oic acid	[39]
17β-Hydroxyandrost-4-en-3-one	*Corynebacterium* spp.	5β-Androstane-3α,17α-diol	[112]
17β-Hydroxyandrost-4-en-3-one	Mixed bacteria	17α-Hydroxy-5β-androstan-3-one	[113]
3α-Hydroxy-5β-cholanoic acid	*Clostridium perfringens*	3β-Hydroxy-5β-cholanoic acid	[144]
3α,7α-Dihydroxy-5β-cholanoic acid	*Clostridium perfringens*	*3β,7α-Dihydroxy-5β-cholanoic acid*	[144]
3α,7α-Dihydroxy-5β-cholanoic acid	*Clostridium perfringens*	3β,7α-Dihydroxy-5β-cholanoic acid	[159]
3α,7α-Dihydroxy-5β-cholanoic acid	*Clostridium absonum* (immobilized)	3α,7β-Dihydroxy-5β-cholanoic acid	[116]
3α,7α-Dihydroxy-5β-cholanoic acid	*Clostridium limosum* isolate F-14	3α,7β-Dihydroxy-5β-cholanoic acid	[115]
3α,12α-Dihydroxy-5β-cholanoic acid	*Pseudomonas putida* D 4014-A 1615	12β-Hydroxyandrost-4-ene-3,17-dione	[89]
3α,7α,12α-Trihydroxy-5β-cholanoic acid	i) *Pseudomonas putida* ATCC-31752 ii) *Pseudomonas* sp. ATCC-31753	7α,12β-Dihydroxyandrosta-1,4-diene-3,17-dione	[93]
3α,7α,12α-Trihydroxy-5β-cholanoic acid	*Clostridium limosum* isolate F-14	3α,7β,12α-Trihydroxy-5β-cholanoic acid	[115]
3α,7α,12α-Trihydroxy-5β-cholanoic acid	*Bacteroides fragilis* and *Bacteroides uniformis* (mixed culture)	i) 3α,7α,12α-Trihydroxy-5α-cholanoic acid ii) 3α,7β,12α-Trihydroxy-5β-cholanoic acid	[117]

		iii) 3α,12α-Dihydroxy-5α-cholanoic acid	
Pregna-4,16-diene-3,20-dione	*Eubacterium* sp. 144	Pregn-5-ene-3,20-dione	[155]
<u>Methoxylation</u>			
3β-Hydroxypregna-5,16-dien-20-one	*Arthrobacter simplex* (IICB-321)	16α-Methoxy-17β-hydroxyandrosta-1,4-dien-3-one	[21,34]
<u>Oxidation</u>			
Androsta-4,6-diene-3,17-dione	*Rhizopus arrhizus* ATCC-11145	17β-Hydroxyandrosta-4,6-diene-3,16-dione	[46]
3-(*N*-Pyrrolideno)-androsta-3,5-dien-17-one	*Rhizopus arrhizus* ATCC-11145	i) Androst-4-ene-3,17-dione ii) Androst-4-ene-3,6,17-trione iii) 6β-Hydroxyandrost-4-ene-3,17-dione iv) 6α-Hydroxyandrost-4-ene-3,17-dione	[46]
Androst-4-ene-3,17-dione	Mutant strain of *Nocardia restricta*	3aα-H-4α-(3′-propionic acid)-5α-hydroxy-7aβ-methylhexahydro-1-indanone-δ-lactone	[160]
D-Homo-5α-androstane-3,17a-dione	*Cunninghamella elegans*	1β,3β-Dihydroxy-D-homo-5α-androstane-7,17a-dione	[43]
3β-Hydroxy-D-homo-5α-androstan-17a-one	*Cunninghamella elegans*	i) 3β-Hydroxy-D-homo-5α-androstane-7,17a-dione ii) 1β,3β-Dihydroxy-D-homo-5α-androstane-7,17a-dione	[43]
3β-Acetoxy-D-homo-5α-androstan-17a-one	*Cunninghamella elgans*	i) 3β-Hydroxy-D-homo-5α-androstane-7,17a-dione ii) 1β,3β-Dihydroxy-D-homo-5α-androstane-7,17a-dione	[43]
11β-Fluoropregn-4-ene-3,20-dione	*Rhizopus stolonifer* ATCC-6227 b	Pregn-4-ene-3,11,20-trione	[48]
21-Acetoxy-17α-hydroxy-pregn-4-ene-3,20-dione	i) *Cunninghamella blakesleena* ii) *Cunninghamella echniulata* iii) *Cunninghamella homothallica* iv) *Cunninghamella japonica*	21-Acetoxy-17α-hydroxypregn-4-ene-3,11,20-trione	[49]
17α,21-Dihydroxypregn-4-ene-3,20-dione	i) *Cunninghamella blakesleena* ii) *Cunninghamella echniulata* iii) *Cunninghamella homothallica* iv) *Cunninghamella japonica*	17α,21-*Dihydroxypregn-4-ene-3*,11,20-*trione*	[49]
3α,7α-Dihydroxy-5β-cholanoic acid	*Pseudomonaas* sp. mutant RAL 8.23	3,7-Dihydroxy-4(5),9(10)-diseco-5,9,17-trioxoandrosta-1(10),2-dien-4-oic acid	[39]
<u>Rearrangement</u>			
i-Cholesterol	*Moraxella* sp.	Cholesterol	[94]
6β-Methoxy-3α,5α-cyclocholestane	*Moraxella* sp.	Cholesterol	[94]

sented in Table 3. Table 4 shows the reductions of keto groups. The miscellaneous reactions, e.g. aromatization, dehydration, epoxidation, hydrogenation, hydrolysis and hydrolysis cum oxidation are presented in Table 5.

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