Phytochemistry, Vol 24, No 7, pp 1403–1421, 1985.
Printed in Great Britain

0031–9422/85 $3 00 + 0.00

REVIEW ARTICLE NUMBER 8

STEROID TRANSFORMATIONS BY MICROORGANISMS—II

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(*Received* 2 *November* 1984)

Key Word Index—Microbial transformation; steroids; cell immobilization.

Abstract—Steroid transformations by various microorganisms are reviewed. Newer developments in this area are discussed. A compilation of a variety of steroid substrates used for transformations by microorganisms during the period 1982–mid 1984 along with the metabolites formed, is included.

INTRODUCTION

Since the discovery in 1952 by Murray and Peterson that the fungus *Rhizopus arrhizus* was able to convert progesterone into its 11α-hydroxy derivative in high isolable yield, microbial transformations have been extensively applied in the production of steroids on a commercial scale. In the preparation of various steroids microbial transformations, *viz.* hydroxylation, dehydrogenation, side chain degradation etc. now constitute an indispensable synthetic arsenal. In a previous review [1] the literature which developed during the period 1979–1981 was covered including citation of earlier monographs and reviews. This review presents the position reached during the period 1982–mid 1984.

Research on the microbial transformation of steroids is being pursued for development of processes for exploitation by the drugs industry and also for preparation of newer potentially useful steroid analogues which are otherwise inaccessible. Evidently, the development of high yielding processes is the ultimate aim. Attempts are made for improvement of strains, modification of substrates and manipulation of media, temperature and period of incubation to achieve this objective. Strain improvement is a complex and multidisciplinary activity and an excellent review on this subject has appeared [2]. Modifications of substrates are made for their facile transformation by microorganisms to the desired products. Employment of appropriate media for optimal yields of products is an essential step and there is sufficient scope for innovative approaches in this area also. Optimization of incubation periods and temperature, however, requires comparatively less effort.

HYDROXYLATION

Every site in the steroid molecule is accessible for microbial hydroxylation. In fact almost all the positions in a steroid molecule have been hydroxylated by various microbial strains. However, microbial hydroxylations which are of considerable commercial importance, particularly 11α- and 16α-hydroxylations leading to corticosteroids and their analogues received greater attention. During the period under review a variety of steroid substrates have been studied for hydroxylation. The substrates and the metabolites are listed in Table 1 in order of the hydroxylation sites.

SIDE CHAIN DEGRADATION

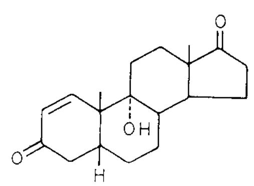
Next to hydroxylation the other microbial reaction which has received considerable attention is the degradation of the C-17 side chain of widely available steroids for the preparation of androstanes obviously due to the fact that a chemical solution to the problem is not economical. A variety of strains and media have been employed for the study. The conversion of cholesterol and sitosterol to degradation products was studied using *Mycobacterium phlei* and *Mycobacterium* NRRL B-3683 and NRRL B-3805 [3]. The medium used was nutrient broth at different glucose concentrations. Testosterone and Δ^1-testosterone were the major products at 4% glucose concentration, whereas if the concentrations were reduced to 1.5% or less the major products were 17-keto steroids. Methods for preparation of 12β-hydroxyandrosta-1,4-diene-3,17-dione from deoxycholic acid or its salts using the strains *Pseudomonas stutzeri* [4], *Aeromonas* [5] and *Pseudomonas ovalis* [6] have been patented. The composition of the media used for the transformation studies was more or less the same. Thus, for *Pseudomonas ovalis* a seed culture of the strain was inoculated into 1 l. medium containing deoxycholic acid (50 g), NH_4NO_3 (2 g), KH_2PO_4 (1 g), K_2HPO_4 (5 g), $MgSO_4 7H_2O$ (0.2 g), yeast extract (0.2 g) and NaOH (5 g) and incubated at 30° for 5 days with shaking. Production of 7α-hydroxyandrosta-1,4-diene-3,17-dione from 3α,7α-dihydroxy-5β-cholanic acid and its salts using a *Pseudomonas* strain has been patented [7]. A *Pseudonocardia* strain has been used for the production of androsta-1,4-diene-3,17-dione and androst-4-ene-3,17-dione in the absence of oxidative inhibitors [8]. For example, *P. methanolignica* DIC-94125 was shake cultured at 40° on a medium (1 l.) containing glucose (20 g), NaH_2PO_4 (3 g), K_2HPO_4 (7 g), peptone (5 g) and cornsteep liquor (5 g). Cholesterol (500 mg in 5 ml MeOH) was added after 48 hr and the culture was shaken for an additional 72 hr. Production of androsta-1,4-diene-3,17-dione and

Table 1 Hydroxylation

Substrate	Microorganism	Product	Reference
1α-Hydroxylation			
3β-Hydroxyandrost-5-en-17-one	(i) *Penicillium oxalicum* FERM-P4729 or (ii) *Aspergillus terrens*	1α,3β-Dihydroxyandrost-5-en-17-one	27
3β-Acetoxyandrost-5-en-17-one	(i) *Penicillium oxalicum* FERM P-4729 or (ii) *Aspergillus terrens*	1α,3β-Dihydroxyandrost-5-en-17-one	27
1β-Hydroxylation			
Pregn-4-ene-3,20-dione	*Aspergillus ochraceus* NRRL-405	1β,6α-Dihydroxypregn-4-ene-3,20-dione	28
5α-Hydroxylation			
16α,17α-Epoxy-5α-androstan-3-one	*Curvularia clavata*	16α,17α-Epoxy-5α-androstane-3α,5,6α-triol	29
16β,17β-Epoxy-5α-androstan-3-one	*Curvularia clavata*	16β,17β-Epoxy-5α-androstane-3β,5,7α-triol	29
6α-Hydroxylation			
Androst-4-ene-3,17-dione	*Mucor* sp.	6,14α-Dihydroxyandrost-4-ene-3-one	26
16α,17α-Epoxy-5α-androstan-3-one	*Curvularia clavata*	16α,17α-Epoxy-5α-androstane-3α,5,6α-triol	29
D-Homo-5α-androstane-2,17a-dione	*Rhizopus nigrican*	6α-Hydroxy-D-homo-5α-androstane-2,17a-dione	30
Pregn-4-ene-3,20-dione	*Aspergillus ochraceus* NRRL-405	1β,6α-Dihydroxypregn-4-ene-3,20-dione	28
6β-Hydroxylation			
Pregn-4-ene-3,20-dione	*Aspergillus niger*	6β-11α-Dihydroxypregn-4-ene-3,20-dione	31
11α-Hydroxypregn-4-ene-3,20-dione	*Aspergillus niger*	6β,11α-Dihydroxypregn-4-ene-3,20-dione	31
7α-Hydroxylation			
16β,17β-Epoxy-5α-androstan-3-one	*Curvularia clavata*	16β,17β-Epoxy-5α-androstane-3β,5,7α-triol	29
7β-Hydroxylation			
D-Homo-5α-androstane-2,17a-dione	*Rhizopus nigrican*	7β-Hydroxy-D-homo-5α-androstane-2,17a-dione	30
D-Homo-5α-androstane-3,17-dione	*Rhizopus nigrican*	3β,7β-Dihydroxy-D-homo-5α-androstan-17-one	30
3β-Hydroxy-D-homo-5α-androstan-17-one	*Rhizopus nigrican*	D-Homo-5α-androstane-3β,7β,17α-triol	30
3β-Hydroxy-D-homo-5α-androstan-17a-one	*Rhizopus nigrican*	3β,7β-Dihydroxy-D-homo-5α-androstan-17a-one	30
3α-Hydroxy-5β-cholanoic acid	(i) *Fusarium equiseti* M-41 or (ii) *Curvularia lunata* ATCC-13432	3α,7β-Dihydroxy-5β-cholanoic acid	32
Pregn-4-ene-3,20-dione	*Aspergillus fumigatus*	7β,15β-Dihydroxypregn-4-ene-3,20 dione	33
9-Hydroxylation			
Androst-4-ene-3,17-dione	*Nocardia conicruria* ATCC-31548	9-Hydroxyandrost-4-ene-3,17-dione	34
3α,12α-Dihydroxy-5β-cholanoic acid	*Pseudomonas* NCIB-10590	9α-Hydroxyandrost-1-ene-3,17-dione	11
6β-Fluorocholest-4-en-3-one	*Mycobacterium fortuitum* NRRL B-8119	6-Fluoro-9α-hydroxyandrost-4-ene-3,17-dione	35
Sitosterol	*Mycobacterium fortuitum*	9-Hydroxypregna-4,17(20)-dien-3-one-20-carboxylic acid	24
	Mycobacterium fortuitum NRRL B-12433	9-Hydroxypregna-4,17(20)-dien-3-one-20-carboxaldehyde	25
11α-Hydroxylation			
D-Homo-5α-androstane-3,17-dione	*Rhizopus nigrican*	3α,11α-Dihydroxy-D-homo-5α-androstan-17-one	30
Cholesterol	*Pseudonocardia methanolignica* DIC 94127 and *Neosartorya fischeri fischeri*	11α-Hydroxyandrost-4-ene-3,17-dione	19
Pregn-4-ene-3,20-dione	*Apergillus fumigatus*	11α,15β-Dihydroxypregn-4-ene-3,20-dione	33
	Rhizopus stolonifer	11α-Hydroxypregn-4-ene-3,20-dione	36
	Aspergillus ochraceus	11α-Hydroxypregn-4-ene-3,20-dione	37
	Aspergillus niger	11α-Hydroxypregn-4-ene-3,20-dione	31

	Aspergillus ochraceus NRRL-405	11α-Hydroxypregn-4-ene-3,20-dione	28
	Aspergillus phoenicis	11α-Hydroxypregn-4-ene-3,20-dione	38
	Bacillus sp.	(i) 11α-Hydroxy-5α-pregnane-3,6,20-trione (ii) 11α,14α-Dihydroxypregn-4-ene-3,20-dione	39
16α,17α-Epoxypregn-4-ene-3,20-dione	*Rhizopus* 80322	16α,17α-Epoxy-11α-hydroxypregn-4-ene-3,20-dione	40
17α,21-Dihydroxypregn-4-ene-3,20-dione	*Cunninghamella elegans*	11α,17α,21-Trihydroxypregn-4-ene-3,20-dione	41
17α,21-Dihydroxy-6α-fluoro-16α-methylpregn-4-ene-3,20-dione	*Metarrhizium velutinum*	6-Fluoro-16α-methyl-11α,17α,21-trihydroxypregn-4-ene-3,20-dione	42
<u>11β-Hydroxylation</u>			
3β,21-Diacetoxy-17-(methoxy methoxy) pregn-5-en-20-one	*Flavobacterium dehydrogenans* and *Curvularia lunata*	11β,21-Dihydroxy-17-(methoxy methoxy)pregn-4-ene-3,20-dione	21
17α,21-Dihydroxypregn-4-ene-3,20-dione	*Cunninghamella elegans*	11β,17α,21-Trihydroxypregn-4-ene-3,20-dione	41
	Curvularia lunata (immobilized)	11β,17α,21-Trihydroxypregn-4-ene-3,20-dione	43
	Pellicularia filamentosa	11β,17α,21-Trihydroxypregn-4-ene-3,20-dione	44
3β,17α,21-Triacetoxypregn-5-en-20-one	*Flavobacterium dehydrogenans* and *Curvularia lunata* NRRL-2380	17α-Acetoxy-11β,21-dihydroxypregn-4-ene-3,20-dione	23
<u>12β-Hydroxylation</u>			
Withaferin 'A'	*Cunninghamella elegans* NRRL-1393	12β-Hydroxywithaferin 'A'	45
<u>14-Hydroxylation</u>			
Androst-4-ene-3,17-dione	*Mucor* sp.	(i) 6,14α-Dihydroxyandrost-4-en-3-one (ii) 14α-Hydroxyandrost-4-ene-3,17-dione	26
16β,17β-Epoxy-5α-androstan-3-one	*Curvularia clavata*	16β,17β-Epoxy-3β,14-dihydroxy-5α-androstan-7-one	29
17β-Hydroxyandrost-4-en-3-one	*Bacillus* sp.	(i) 14α-Hydroxyandrost-4-ene-3,17-dione (ii) 14α,17β-Dihydroxyandrost-4-en-3-one	39
Pregn-4-ene-3,20-dione	*Bacillus* sp.	(i) 14α-Hydroxypregn-4-ene-3,20-dione (ii) 14α-Hydroxy-5α-pregnane-3,6,20-trione (iii) 11α,14α-Dihydroxypregn-4-ene-3,20-dione	39
<u>15β-Hydroxylation</u>			
16β,17β-Epoxy-5α-androstan-3-one	*Curvularia clavata*	16β,17β-Epoxy-5α-androstane-3α,15β-diol	29
17β-Hydroxyandrost-4-en-3-one	*Aspergillus fumigatus*	15β,17β-Dihydroxyandrost-4-en-3-one	46
Pregn-4-ene-3,20-dione	*Aspergillus fumigatus*	(i) 15β-Hydroxypregna-1,4-diene-3,20-dione (ii) 7β,15β-Dihydroxypregn-4-ene-3,20-dione (iii) 11α,15β-Dihydroxypregn-4-ene-3,20-dione	33
Withaferin 'A'	*Cunninghamella elegans* NRRL-1393	15β-Hydroxywithaferin 'A'	45
<u>16α-Hydroxylation</u>			
3β-Hydroxyandrost-5-en-17-one	*Streptomyces roseochromogenes* (Immobilized)	3β,16α-Dihydroxyandrost-5-en-17-one	18
<u>17α-Hydroxylation</u>			
D-Homo-5α-androstane-3,6-dione	*Rhizopus nigrican*	3β,17α-Dihydroxy-D-homo-5α-androstan-6-one	30
D-Homo-5α-androstane-3,7-dione	*Rhizopus nigrican*	(i) 17α-Hydroxy-D-homo-5α-androstane-3,7-dione (ii) 3β,17α-Dihydroxy-D-homo-5α-androstan-7-one	30
<u>19-Hydroxylation</u>			
17α,21-Dihydroxypregn-4-ene-3,20-dione	*Pellicularia filamentosa*	17α,19,21-Trihydroxypregn-4-ene-3,20-dione	44
<u>26-Hydroxylation</u>			
Cholesterol	*Pseudomonas* NCIB-10590	26-Hydroxycholest-4-en-3-one	47

androst-4-ene-3,17-dione from cholesterol using the strain *P. methanolignica* LM-145 (FERM-P 6057) in the presence of an inhibitor has also been patented. The strain was inoculated in 100 ml media (pH 7.0) containing (per litre) glucose (10 g), NH_4Cl (4 g), K_2HPO_4 (7 g), NaH_2PO_4 $2H_2O$ (3 g), $MgSO_4$ (0.5 g), yeast extract (0.03 g), peptone (1 g) and trace amounts of $FeCl_3$, $CuSO_4$, $ZnSO_4$, $MnSO_4$ and $CoCl_2$ and cultured with shaking at 40° for 18 hr. The broth was mixed with 100 mg cholesterol suspended in 2 ml H_2O and further cultured for 6 hr. Then the broth was mixed with 15.62 mg 2,2′-dipyridyl dissolved in 0.1 ml MeOH and further cultured for 42 hr [9]. The ability of *Aspergillus aureofulgens* to cleave the side chain of progesterone and related C-21 steroids was studied. The cleavage of the side chain of progesterone and related compounds was followed by the stereospecific reduction of the generated androst-4-ene-3,17-dione to the 5β-androstane derivatives. Both the oxygenase and reductase activities seemed to be influenced by the growth conditions [10]. The anaerobic metabolism of deoxycholic acid by *Pseudomonas* NCIB 10590 was reported [11]. The metabolic pathway was similar to that operating under aerobic conditions with 12β-hydroxyandrosta-1,4-diene-3,17-dione as the major neutral product and 12α-hydroxy-5β-cholan-3-oxo-24-oic acid as the major acidic product. Four other metabolites which are not produced during aerobic metabolism were isolated and characterized as 9α-hydroxyandrost-1-ene-3,17-dione (**1**), 12α,17β-dihydroxyandrosta-1,4-dien-3-one (**2**), 3β,12β-dihydroxy-5β-androstan-17-one (**3**) and 3-hydroxy-9,10-seco-1,3,5(10)-androstatriene-9,17-dione (**4**). The preparation of 24-nor-1,4-choladiene-3,22-dione (**5**) from sitosterol by fermentation with *Mycobacterium fortuitum* has been patented. A seed culture of the microorganism was inoculated into a medium (pH 7.0) containing (per litre) ucon (8 g), cerelose (5 g), NH_4Cl (3 g), $CaCO_3$ (3 g), trisodium citrate dihydrate (3 g), tween 80 (2 g), soy flour (1 g), KH_2PO_4 (0.5 g), urea (0.5 g) and sitosterol (30 g) and incubated at 31° for 7 days with shaking. The broth was then worked up for isolation of the metabolite [12]. The degradation of the C-17 side chain of Reichstein substance S leading to the formation of androst-4-ene-3,17-dione has been achieved [13] by fermentation with a strain of *Bacillus* sp. in a



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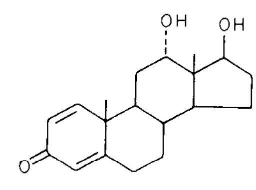

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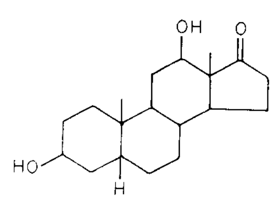

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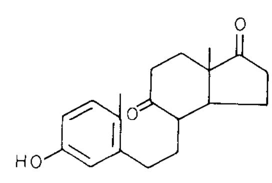

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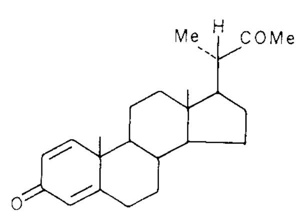

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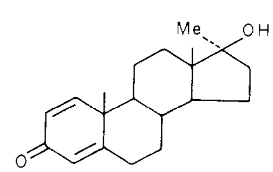

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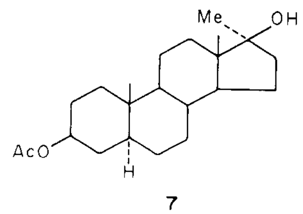

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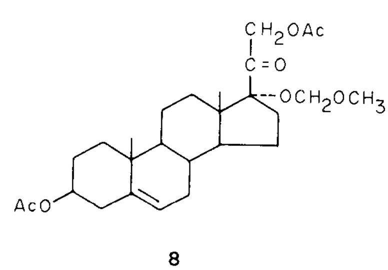

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medium (pH 7.0) containing (per litre) peptone (5 g), beef extract (2.5 g), yeast extract (2.5 g) sodium chloride (2.5 g) and substance 'S' (500 mg) at 37° for 72 hr with shaking.

IMMOBILIZATION OF MICROBIAL CELLS

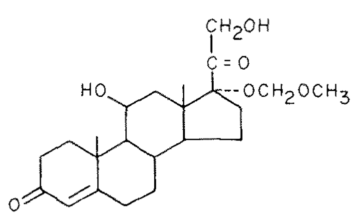
The utilization of immobilized enzymes and cells for steroid transformations has received much attention in recent years. A variety of methods for immobilization of enzymes have been developed and more than one hundred immobilization techniques have been elaborated [14, 15]. A general method of immobilization of cells with preserved viability has been described [16].

The effect of polymerization conditions and polyacrylamide gel structure on the sterol metabolizing enzyme of immobilized *Mycobacterium phlei* 1026 cells was studied using 4-cholesten-3-(*O*-carboxymethyl)-oxime as a substrate. Optimum conditions for immobilization and sterol transformation were (i) cell incorporation into 10% gel at a monomer ratio (acrylamide–methylene bis-acrylamide) of 95:5, (ii) polymerization temperature 2–8°, (iii) a biomass concentration of 40 mg/ml and (iv) granule size approximately 1.0 mm. The enzymic activity was substantially increased when the immobilized cells were incubated in a nutrient medium [17].

The method of 16α-hydroxylation of steroids with gel entrapped *Streptomyces roseochromogenes* cells in a water–organic cosolvent system has been patented [18]. Cells of *S. roseochromogenes* NRRL-B-1233 were immobilized in photo-crosslinked BPE-30 resin. The immobilized microorganism catalysed the transformation of dehydroepiandrosterone to 16α-hydroxydehydroepiandrosterone. The original activity of the free cells was almost 100% retained after immobilization. The stability of the steroid 16α-hydroxylase activity of the cells over repeated reactions was greatly improved by immobilization. Thus the usefulness of the BPE-30 hydrophilic resin in immobilization of *S. roseochromogenes* having steroid 16α-hydroxylation activity was demonstrated.

MIXED CULTURE FERMENTATION AND OTHER NEWER TECHNIQUES

It has been demonstrated that microbial two step conversions may be effected by mixed culture fermentation. In an experiment *Pseudonocardia methanolignica* DIC 94127 was cultured with shaking at 40° for 48 hr on a medium containing (per litre) glucose (20 g), cornsteep liquor (10 g), peptone (5 g), NaH_2PO_4 (3 g) and K_2HPO_4 (7 g). Cholesterol (500 mg in 5 ml MeOH) was added to 100 ml medium. The mixture was shaken at 40° for 128 hr and then inoculated with *Neosartorya fischeri fischeri* culture and further incubated at 30° for 96 hr. A good yield of 11α-hydroxyandrost-4-ene-3,17-dione was thus obtained [19]. 17α-Methyl-17β-hydroxyandrosta-1,4-dien-3-one (**6**) was prepared from **7** by fermentation with



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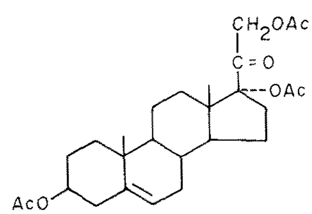

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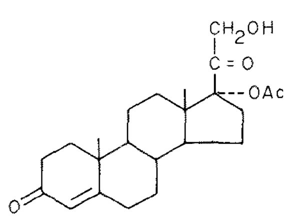

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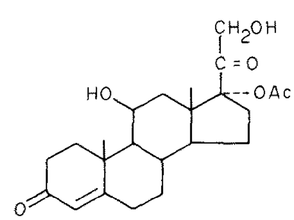

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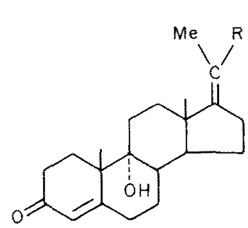

13 R=COOH

14 R=CHO

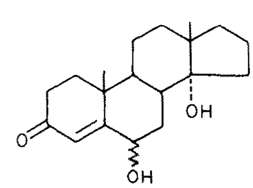

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Table 2. Dehydrogenation

Substrate	Microorganism	Product	Reference
3β-Acetoxy-17α-methyl-5α-androstan-17β-ol	*Nocardia* 76816 and *Arthrobacter simplex* A-1 (mixed culture)	17β-Hydroxy-17α-methylandrosta-1,4-dien-3-one	20
3β-Hydroxyandrost-5-en-17-one	(i) *Fusarium solani* or (ii) *Flavobacterium dehydrogenans*	Androst-4-ene-3,17-dione	37
6β-Hydroxyandrost-4-ene-3,17-dione	*Caldariella acedophila*	5α-Androstane-3,6,17-trione	48
17β-Hydroxyandrost-4-en-3-one	*Aspergillus aureogulgens*	(i) Androst-4-ene-3,17-dione	10
		(ii) 5β-Androstane-3,17-dione	
	Bacillus sp.	(i) Androst-4-ene-3,17-dione	39
		(ii) 14α-Hydroxyandrost-4-ene-3,17-dione	
3α,6α-Dihydroxy-5β-cholanoic acid	*Pseudomonas* NCIB 10590	(i) 6α-Hydroxychol-4-en-3-one-24-oic acid	49
		(ii) Chola-1,4-dien-3-one-24-oic acid	
		(iii) 6α-Hydroxychola-1,4-dien-3-one-24-oic-acid	
		(iv) 6α-Hydroxyandrosta-1,4-diene-3,17-dione	
		(v) Androst-4-ene-3,17-dione	
		(vi) Androst-4-ene-3,6,17-trione	
		(vii) Androsta-1,4-diene-3,6,17-trione	
		(viii) Androsta-1,4-diene-3,17-dione	
		(ix) 17β-Hydroxyandrosta-1,4-dien-3-one	
		(x) 6α-Hydroxyandrost-4-ene-3,17-dione	
		(xi) 3-Oxochol-5-en-24-oic acid	
		(xii) 3-Oxochol-4-en-24-oic acid	
		(xiii) Androst-4-ene-3,6,17-trione	
		(xiv) Androsta-1,4-diene-3,6,17-trione	
	Clostridium perfringens	7α-Hydroxy-3-oxo-5β-cholanoic acid	50
	Pseudomonas NCIB-10590	7α-Hydroxy-3-oxo-5β-cholanoic acid	51
	Pseudomonas brevis CD-21	7α-Hydroxyandrosta-1,4-diene-3,17-dione	7
	Pseudomonas NCIB-10590	(i) 3-Oxo-chola-4,6-diene-24-oic acid	51
		(ii) Pregna-4,6-diene-3-one-20-carboxylic acid	
		(iii) 7α-Hydroxypregna-1,4-dien-3-one-20-carboxylic acid	
		(iv) 7α-Hydroxypregn-4-en-3-one-20-carboxylic acid	
		(v) Androsta-1,4,6-triene-3,17-dione	
		(vi) Androsta-4,6-diene-3,17-dione	
		(vii) 17β-Hydroxyandrosta-4,6-dien-3-one	
		(viii) 17β-Hydroxyandrosta-1,4,6-trien-3-one	
		(ix) 7α-Hydroxyandrosta-1,4-diene-3,17-dione	
		(x) 7α-Hydroxyandrost-4-ene-3,17-dione	
	Clostridium absonum	3α-Hydroxy-7-oxo-5β-cholanoic acid	52
3α,12α-Dihydroxy-5β-cholanoic acid	(i) *Pseudomonas arvilla* or (ii) *Alcaligenes faecalis*	12α-Hydroxypregna-1,4-dien-3-one-20α-carboxylic acid	53

	Pseudomonas stutzeri D-297	12β-Hydroxyandrosta-1,4-diene-3,17-dione	4
	Aeromonas hydrophila D-283	12β-Hydroxyandrosta-1,4-diene-3,17-dione	5
	Pseudomonas ovalis D-40	12β-Hydroxyandrosta-1,4-diene-3,17-dione	6
	Alcaligenes faecalis FERM BP-204	12α-Hydroxypregna-1,4-dien-3-one-20-carbaldehyde	54
	Pseudomonas NCIB-10590	(i) 9α-Hydroxy-5β-androst-1-ene-3,17-dione	11
		(ii) 12α,17β-Dihydroxyandrosta-1,4-dien-3-one	
		(iii) 12α-Hydroxy-3-oxo-5β-cholanoic acid	
3α,7α,12α-Trihydroxy-5β-cholanoic acid	*Pseudomonas* NCIB 10590	(i) 12α-Hydroxyandrosta-4,6-diene-3,17-dione	55
		(ii) 12α-Hydroxyandrosta-1,4-diene-3,17-dione	
		(iii) Chola-4,6-diene-3,12-dione-24-oic acid	
		(iv) 12α-Hydroxypregna-4,6-dien-3-one-20-carboxylic acid	
		(v) 12α-Hydroxychola-4,6-diene-3-one-24-oic acid	
		(vi) 7α,12α-Dihydroxy-3-oxo-5β-cholanoic acid	
		(vii) 12α-Hydroxy-5β-androstane-3,17-dione	
	Clostridium perfringens	7α,12α-Dihydroxy-3-oxo-5β-cholanoic acid	50
	Corynebacterium CA-281	(i) 3α,12α-Dihydroxy-7-oxo-5β-cholanoic acid	56
		(ii) 3α,12α-Dihydroxy-7-oxo-5β-bisnorcholanoic acid	
	Nocardia CA-173	3α,7α-Dihydroxy-12-oxo-5β-cholanoic acid	57
	Pseudomonas sp. NCIB 10590	3,12-Dioxo-cholan-4,6-dien-24-oic acid	55
Cholesterol	*Arthrobacter simplex* ATCC-6946	Androsta-1,4-diene-3,17-dione	58
	Arthrobacter sp.	(i) Androsta-1,4-diene-3,17-dione	59
		(ii) Cholesta-1,4-dien-3-one	
	(i) *Mycobacterium phlei* or (ii) *Mycobacterium* NRRL B-3683 or (iii) *Mycobacterium* NRRL B-3805	17β-Hydroxyandrosta-1,4-dien-3-one	3
	Pseudonocardia methanolignica LM-145 FERM-P 6057	Androsta-1,4-diene-3,17-dione	9
	Mycobacterium aurum	Pregna-1,4-diene-3,20-dione	60
	Pseudonocardia methanolignica DIC-94125	Androsta-1,4-diene-3,17-dione	8
	Pseudonocardia methanolignica DIC-94125	(i) Cholesta-1,4-dien-3-one	61
		(ii) Androsta-1,4-diene-3,11-dione	
	Pseudomonas NCIB-10590	(i) Pregna-1,4-dien-3-one-20-carboxylic acid	47
		(ii) Androsta-1,4-diene-3,17-dione	
	Arthrobacter sp.	(i) Androst-4-ene-3,17-dione	59
		(ii) Cholest-4-en-3-one	
	(i) *Mycobacterium phlei* or (ii) *Mycobacterium* NRRL B-3683 or (iii) *Mycobacterium* NRRL B-3805	17β-Hydroxyandrost-4-en-3-one	3
	Pseudonocardia methanolignica LM-145 FERM-P-6057	Androst-4-ene-3,17-dione	9
	Mycobacterium aurum	Pregn-4-ene-3,20-dione	60
	Pseudonocardia methanolignica DIC-94125	Androst-4-ene-3,17-dione	8
	Pseudonocardia methanolignica DIC-94125	(i) Cholest-4-en-3-one	61
		(ii) Androst-4-ene-3,11-dione	

Table 2 (*Continued*)

Substrate	Microorganism	Product	Reference
	Pseudomonas NCIB-10590	(i) Cholest-5-en-3-one	47
		(ii) Cholest-4-en-3-one	
		(iii) Cholest-4-en-3-one-21-carboxylic acid	
		(iv) 3-Oxo-chol-4-en-24-oic acid	
		(v) Pregn-4-ene-3-one-20-carboxylic acid	
		(vi) 26-Hydroxycholest-4-en-3-one	
	Pseudonocardia methanolignica DIC-94127 and *Neosartorya fischeri fischeri*	11α-Hydroxyandrost-4-ene-3,17-dione	19
Cholest-5-ene-3β,19-diol	(i) *Mycobacterium* NRRL B-3805 or (ii) *Mycobacterium phlei*	19-Hydroxyandrost-4-ene-3,17-dione	62
16α,17α-Epoxy-5α-pregnan-3β-ol-20-one	*Nocardia* sp.	16α,17α-Epoxypregn-4-ene-3,20-dione	63
16α,17α-Epoxy-5β-pregnan-3β-ol-20-one	*Nocardia* sp.	16α,17α-Epoxypregn-4-ene-3,20-dione	63
16α,17α-Epoxy-5α-pregnan-3β-ol-20-one	*Arthrobacter simplex*	(i) 16α,17α-Epoxypregna-1,4-dien-20-ol-3-one	63
		(ii) 16α,17α-Epoxypregn-4-en-20α-ol-3-one	
16α,17α-Epoxy-16β-methyl-5α-pregn-9(11)-en-3β-ol-20-one	*Nocardia* sp.	(i) 16α,17α-Epoxy-16β-methylpregna-4,9(11)-diene-3,20-dione	63
		(ii) 16α,17α-Epoxy-16β-methylpregna-1,4,9(11)-triene-3,20-dione	
Pregn-4-ene-3,20-dione	*Aspergillus fumigatus*	15β-Hydroxypregna-1,4-diene-3,20-dione	33
3-Acetoxy-5α-pregn-16-en-20-one	*Arthrobacter* 9-2	Androsta-1,4-diene-3,17-dione	64
11β,17α,21-Trihydroxypregn-4-ene-3,20-dione	(i) *Mycobacterium strain* 462 or (ii) *Arthrobacter simplex* 1652	11β,17α,21-Trihydroxypregna-1,4-diene-3,20-dione	65
11β,17α,21-Trihydroxypregn-4-ene-3,20-dione	*Arthrobacter simplex*	11β,17α,21-Trihydroxypregna-1,4-diene-3,20-dione	22
11β,17α,21-Trihydroxypregn-4-ene-3,20-dione	*Corynebacterium simplex* (immobilized)	11β,17α,21-Trihydroxypregna-1,4-diene-3,20-dione	66
17α-Acetoxy-11β,21-dihydroxypregn-4-ene-3,20-dione	*Arthrobacter simplex* ATCC-6946	17α-Acetoxy-11β,21-dihydroxypregna-1,4-diene-3,20-dione	23
21-Acetoxy-17α-hydroxy-16α-methylpregna-4,9(11)-diene-3,20-dione	*Arthrobacter simplex*	17α,21-Dihydroxy-16α-methylpregna-1,4,9(11)-triene-3,20-dione	67
21-Acetoxy-3β,17α-dihydroxy-16β-methyl-5α-pregn-9(11)-en-20-one	*Arthrobacter* 9-2	17α,21-Dihydroxy-16β-methylpregna-1,4,9(11)-triene-3,20-dione	68
3α-Hydroxy-5β-pregnan-20-one	*Aspergillus aureogulgens*	5β-Androstane-3,17-dione	10
16α,17α-Epoxy-5α-pregnan-3β-ol-20-one	*Arthrobacter simplex*	16α,17α-Epoxy-20α-hydroxypregna-1,4-diene-3-one	63
16α,17α-Epoxy-16β-methyl-5α-pregn-9(11)-en-3β-ol-20-one	*Nocardia* sp.	16α,17α-Epoxy-16β-methyl-5α-pregn-9(11)-ene-3,20-dione	63
Pregn-5-ene-3β-ol-20-one	*Aspergillus aureogulgens*	(i) Androst-4-ene-3,17-dione	16
		(ii) 17β-Hydroxy-androst-4-ene-3-one	
		(iii) Testolactone	
21-Acetoxy-3β,17α-dihydroxy-16β-methyl-5α-pregn-9(11)-en-20-one	*Arthrobacter* 9-2	17α,21-Dihydroxy-16β-methylpregna-1,4,9(11)-triene-3,20-dione	68
6β-Hydroxypregn-4-ene-3,20-dione	*Caldariella acidophila*	(i) Pregn-4-ene-3,6,20-trione	48

		(ii) 5α-Pregnane-3,6,20-trione	
Sitosterol	*Arthrobacter oxydans*	Androsta-1,4-diene-3,17-dione	69, 70
	Coryneform sp. ATCC-31458	Pregna-1,4-dien-3-one-20-carboxylic acid	71
	Arthrobacter sp.	Androsta-1,4-diene-3,17-dione	59
	(i) *Mycobacterium phlei* or (ii) *Mycobacterium* NRRL B-3683 or (iii) *Mycobacterium* NRRL B-3805	17β-Hydroxyandrosta-1,4-dien-3-one	3
	Mycobacterium fortuitum	24-Nor-chola-1,4-diene-3,22-dione	12
	Coryneform sp. ATCC-31485	Pregn-4-en-3-one-20-carboxylic acid	71
	Arthrobacter sp.	(i) Androst-4-ene-3,17-dione (ii) Stigmast-4-en-3-one	59
	Mycobacterium fortuitum	9-Hydroxypregna-4,17(20)-diene-3-one-20-carboxylic acid	24
	(i) *Mycobacterium phlei* or (ii) *Mycobacterium* NRRL-B-3683 or (iii) *Mycobacterium* NRRL-B-3805	17β-Hydroxyandrost-4-en-3-one	3
	(i) *Mycobacterium phlei* or (ii) *Mycobacterium* NRRL B-3683 (iii) *Mycobacterium* NRRL B-3805	17β-Hydroxyandrosta-1,4-dien-3-one	3
	Arthrobacter oxydans	Androsta-1,4-diene-3,17-dione	70
Sitost-5-ene-3β,19-diol	(i) *Mycobacterium* NRRL-B-3805 or (ii) *Mycobacterium phlei*	19-Hydroxyandrost-4-ene-3,17-dione	62
Sitosterol	*Mycobacterium fortuitum*	24-Nor-chola-1,4-diene-3,22-dione	12
	Mycobacterium NRRL B-3805	Androst-4-ene-3,17-dione	72

Table 3. Side chain degradation

Substrate	Microorganism	Product	Reference
3α,12α-Dihydroxy-5β-cholanoic acid	DSM-1444	(i) 12-Oxo-bisnor-5β-cholanoic acid	73
		(ii) 12α-Hydroxy-5β-bisnor-cholanoic acid	
	(i) *Pseudomonas arvilla* or	12α-Hydroxypregna-1,4-dien-3-one-20α-carboxylic acid	53
	(ii) *Alcaligenes faecalis*		
	Pseudomonas stutzeri D-297	12β-Hydroxyandrosta-1,4-diene-3,17-dione	4
	Aeromonas hydrophila D-283	12β-Hydroxyandrosta-1,4-diene-3,17-dione	5
	Pseudomonas ovalis D-40	12β-Hydroxyandrosta-1,4-diene-3,17-dione	6
3α,7α,12α-Trihydroxy-5β-cholanoic acid	*Corynebacterium* CA-281	3α,12α-Dihydroxy-7-oxo-5β-bisnor-cholanoic acid	56
3α,7α-Dihydroxy-5β-cholanoic acid	*Pseudomonas brevis* CD-21	7α-Hydroxyandrosta-1,4-diene-3,17-dione	7
	Pseudomonas NCIB-10590	(i) 3-Oxopregna-4,6-diene-20-carboxylic acid	51
		(ii) 7α-Hydroxypregna-1,4-diene-3-one-20-carboxylic acid	
		(iii) 7α-Hydroxypregna-4-en-3-one-20-carboxylic acid	
		(iv) Androsta-4,6-diene-3,17-dione	
		(v) Androsta-1,4,6-triene-3,17-dione	
		(vi) 17β-Hydroxyandrosta-4,6-dien-3-one	
		(vii) 17β-Hydroxyandrosta-1,4,6-trien-3-one	
		(viii) 7α-Hydroxyandrosta-1,4-diene-3,17-dione	
		(ix) 7α-Hydroxy-androst-4-ene-3,17-dione	
3α,6α-Dihydroxy-5β-cholanoic acid	*Pseudomonas* NCIB-10590	(i) 6α-Hydroxyandrosta-1,4-diene-3,17-dione	49
		(ii) Androst-4-ene-3,17-dione	
		(iii) Androst-4-ene-3,6,17-trione	
		(iv) Androsta-1,4-diene-3,6,17- rione	
		(v) Androsta-1,4-diene-3,17-dione	
		(vi) 17β-Hydroxyandrosta-1,4-dien-3-one	
		(vii) 6α-Hydroxyandrost-4-ene-3,17-dione	
3α,12α-Dihydroxy-5β-cholanoic acid	*Alcaligenes faecalis* FERM BP-204	12α-Hydroxypregna-1,4-dien-3-one-20-carbaldehyde	54
3α,7α,12α-Trihydroxy-5β-cholanoic acid	*Pseudomonas* NCIB 10590	(i) 12α-Hydroxyandrosta-4,6-diene-3,17-dione	55
		(ii) 12α-Hydroxyandrosta-1,4-diene-3,17-dione	
		(iii) 12α-Hydroxypregna-4,6-dien-3-one-20-carboxylic acid	
		(iv) 12α-Hydroxy-5β-androstane-3,17-dione	
		(v) 3α,12α-Dihydroxy-5β-androstan-17-one	
		(vi) 5β-Androstane-3α,12α-17β-triol	
3α,12α-Dihydroxy-5β-cholanoic acid	*Pseudomonas* NCIB 10590	(i) 9α-Hydroxyandrost-1-ene-3,17-dione	11
		(ii) 12α,17β-Dihydroxyandrosta-1,4-diene-3-one	
		(iii) 3-Hydroxy-9,10-seco-1,3,5(10)-androsta triene-9,17-dione	
Cholesterol	*Arthrobacter simplex* ATCC-6946	Androsta-1,4-diene-3,17-dione	58
	Arthrobacter sp.	(i) Androst-4-ene-3,17-dione	59
		(ii) Androsta-1,4-diene-3,17-dione	
	(i) *Mycobacterium phlei* or	17β-Hydroxyandrost-4-en-3-one	3
	(ii) *Mycobacterium* NRRL B-3683 or		
	(iii) *Mycobacterium* NRRL B-3805		

	(i) *Mycobacterium phlei* or	17β-Hydroxyandrosta-1,4-dien-3-one	3
	(ii) *Mycobacterium* NRRL B-3683 or		
	(iii) *Mycobacterium* NRRL-B-3805		
	Pseudonocardia methanolignica LM-145	(i) Androsta-1,4-diene-3,17-dione	9
	(FERM-P6057)	(ii) Androst-4-ene-3,17-dione	
	Mycobacterium aurum	(i) Pregn-4-ene-3,20-dione	60
		(ii) Pregna-1,4-diene-3,20-dione	
	Pseudonocardia methanolignica	(i) Androsta-1,4-diene-3,17-dione	8
	DIC-94125	(ii) Androst-4-ene-3,17-dione	
	Pseudonocardia methanolignica	(i) Androsta-1,4-diene-3,11-dione	61
	DIC-94125	(ii) Androst-4-ene-3,11-dione	
	Pseudomonas NCIB-10590	(i) 3-Oxo-chol-4-ene-24-oic acid	47
		(ii) Pregn-4-en-3-one-20-carboxylic acid	
		(iii) Pregna-1,4-dien-3-one-20-carboxylic acid	
		(iv) Androsta-1,4-diene-3,17-dione	
	Pseudonocardia methanolignica	11α-Hydroxyandrost-4-ene-3,17-dione	19
	DIC-94127 & *Neosartorya fischeri fischeri*		
3-Oxo-24-methylcholest-4-ene-26-oic acid	*Mycobacterium* NRRL-B-3805	(i) 3-Oxochol-4-en-24-oic acid	74
		(ii) 26,27-Bisnorcholest-4-ene-3,24-dione	
		(iii) Androst-4-ene-3,17-dione	
6β-Fluorocholest-4-en-3-one	*Mycobacterium fortuitum* NRRL-B-8119	6-Fluoro-9α-hydroxyandrost-4-ene-3,17-dione	35
6β,19-Oxidocholest-4-en-3-one	(i) *Mycobacterium* NRRL B-3805 or	6β,19-Oxidoandrost-4-ene-3,17-dione	62
	(ii) *Mycobacterium phlei*		
Cholest-5-ene-3β,19-diol	(i) *Mycobacterium* NRRL B-3805 or	19-Hydroxyandrost-4-ene-3,17-dione	62
	(ii) *Mycobacterium phlei*		
	Mycobacterium phlei	(i) Estrone	62
		(ii) Estradiol	
3β-Acetoxy-5α-pregn-16-ene-20-one	*Arthrobacter* 9-2	Androsta-1,4-diene-3,17-dione	64
Pregn-4-ene-3,20-dione	*Aspergillus aureogulgens*	(i) Androst-4-ene-3,17-dione	10
		(ii) 17β-Hydroxyandrost-4-ene-3-one	
		(iii) 5β-Androstane-3,17-dione	
		(iv) Testolactone	
		(v) 17β-Acetoxyandrost-4-en-3-one	
		(vi) 3α-Hydroxy-5β-androstan-17-one	
Pregna-4,16-diene-3,20-dione	*Aspergillus aureogulgens*	(i) Androst-4-ene-3,17-dione	10
		(ii) 17β-Hydroxyandrost-4-en-3-one	
		(iii) Testolactone	
3β-Hydroxypregn-5-en-20-one	*Aspergillus aureogulgens*	(i) Androst-4-ene-3,17-dione	10
		(ii) 17β-Hydroxyandrost-4-en-3-one	
		(iii) Testolactone	
3β-Acetoxypregn-5-en-20-one	*Aspergillus aureogulgens*	(i) Androst-4-ene-3,17-dione	10
		(ii) 17β-Hydroxyandrost-4-en-3-one	
		(iii) Testolactone	
5β-Pregnane-3,20-dione	*Aspergillus aureogulgens*	(i) 5β-Androstane-3,17-dione	10
		(ii) 3α-Hydroxy-5β-androstan-17-one	

Table 3. (*Continued*)

Substrate	Microorganism	Product	Reference
3α-Hydroxy-5β-Pregnan-20-one	*Aspergillus aureogulgens*	(i) 5β-Androstane-3,17-dione (ii) 3α-Hydroxy-5β-androstan-17-one	10
20β-Hydroxypregn-4-en-3-one	*Aspergillus aureogulgens*	(i) Androst-4-ene-3,17-dione (ii) 17β-Hydroxyandrost-4-ene-3,17-dione (iii) Testolactone	10
Sitosterol	*Coryneform* sp. ATCC-31458	(i) Pregn-4-en-3-one-20-carboxylica acid (ii) Pregna-1,4-dien-3-one-20-carboxylic acid	71
	Arthrobacter sp.	(i) Androst-4-ene-3,17-dione (ii) Androsta-1,4-diene-3,17-dione	59
	Arthrobacter oxydans	Androsta-1,4-diene-3,17-dione	69
	Mycobacterium fortuitum	9-Hydroxypregna-4,17(20)-dien-3-one-20-carboxylic acid	24
	(i) *Mycobacterium phlei* or (ii) *Mycobacterium* NRRL-B-3683 or (iii) *Mycobacterium* NRRL-B-3805	17β-Hydroxyandrost-4-en-3-one	3
	(i) *Mycobacterium phlei* or (ii) *Mycobacterium* NRRL B-3683 or (iii) *Mycobacterium* NRRL B-3805	17β-Hydroxyandrosta-1,4-dien-3-one	3
	Mycobacterium fortuitum NRRL B-12433	9-Hydroxypregna-4,17(20)-dien-3-one-20-carboxaldehyde	25
	Arthrobacter oxydans	Androsta-1,4-diene-3,17-dione	70
	Mycobacterium NRRL B-3805	Androst-4-ene-3,17-dione	72
6β,19-Oxidositost-4-en-3-one	(i) *Mycobacterium* NRRL B-3805 or (ii) *Mycobacterium phlei*	6β,19-Oxidoandrost-4-ene-3,17-dione	62
Sitost-5-en-3β,19-diol	(i) *Mycobacterium phlei* or (ii) *Mycobacterium* NRRL-B-3805	19-Hydroxyandrost-4-ene-3,17-dione	62
Sitost-5-ene-3β,19-diol	*Mycobacterium phlei*	(i) Estrone (ii) Estradiol	62
Sitosterol	*Mycobacterium fortuitum*	24-Norchola-1,4-diene-3,22-dione	12

Table 4. Reduction of keto group

Substrate	Microorganism	Product	Reference
2C=O → 2α-OH			
D-Homo-5α-androstane-2,17a-dione	*Rhizopus nigrican*	2α-Hydroxy-D-homo-5α-androstan-17a-one	30
3C=O → 3α-OH			
5α-Androstane-3,17-dione	*Rhodotorula mucilaginosa*	3α-Hydroxy-5α-androstan-17-one	75
5β-Androstane-3,17-dione	*Rhodotorula mucilaginosa*	3α-Hydroxy-5β-androstan-17-one	75
17β-Hydroxy-5α-androstan-3-one	*Rhodotorula mucilaginosa*	5α-Androstane-3α,17β-diol	75
17β-Hydroxy-5β-androstan-3-one	*Rhodotorula mucilaginosa*	5β-Androstane-3α,17β-diol	75
16β,17β-Epoxy-5α-androstan-3-one	*Curvularia clavata*	16β,17β-Epoxy-5α-androstan-3α,15β-diol	29
D-Homo-5α-androstane-3,6-dione	*Rhizopus nigrican*	3α-Hydroxy-D-homo-5α-androstan-6-one	30
D-Homo-5α-androstane-3,17a-dione	*Rhizopus nigrican*	3α-Hydroxy-D-homo-5α-androstan-17a-one	30
D-Homo-5α-androstane-3,17-dione	*Rhizopus nigrican*	(i) 3α-Hydroxy-D-homo-5α-androstan-17-one	30
		(ii) 3α,11α-Dihydroxy-D-homo-5α-androstan-17-one	
Androst-4-ene-3,17-dione	*Aspergillus aureogulgens*	3α-Hydroxy-5β-androstan-17-one	10
18-Hydroxyandrost-4-en-3-one-	*Clostridium paraputrificum*	3α,18-Dihydroxy-5β-androstane-	76
17β-carboxylic acid lactone		17β-carboxylic acid lactone	
17α-Cyanomethylester-4-en-17β-ol-3-one	*Clostridium paraputrificum*	17α-Cyanomethyl-5β-estrane-3α,17β-diol	77
17α-Cyanomethylestr-4-en-17β-ol-3-one	(i) *Rhodosporidium rubrum* or	17α-Cyanomethyl-5α-estrane-3α,17β-diol	77
	(ii) *Rhodotorula glutinis*		
17α-Cyanomethylestra-4,9-dien-17β-ol-3-one	*Clostridium paraputrificum*	17α-Cyanomethylester-9-ene-3α,17β-diol	77
5β-Pregnane-3,20-dione	*Aspergillus aureogulgens*	3α-Hydroxy-5β-androstan-17-one	10
Pregn-4-ene-3,20-dione	*Aspergillus aureogulgens*	3α-Hydroxy-5β-androstan-17-one	10
21-Acetoxy-11β,18-oxidopregn-4-ene-3,20-dione	*Clostridium paraputrificum*	21-Acetoxy-3α-hydroxy-11β,18-oxido-5β-pregnane-20-one	78
11β,17α-Dihydroxypregn-4-ene-3,20-dione	*Clostridium paraputrificum*	3α,11β,17α-Trihydroxy-5β-pregnan-20-one	79
18,21-Dihydroxypregn-4-ene-3,20-dione	*Clostridium paraputrificum*	3α,18,21-Trihydroxy-5β-pregnan-20-one	76
11β,17α,20-Trihydroxypregn-4-en-3-one	*Clostridium paraputrificum*	5β-Pregnane-3α,11β,17α,20-tetrol	79
11β,17α,21-Trihydroxypregn-4-ene-3,20-dione	*Clostridium paraputrificum*	3α,11β,17α,21-Tetrahydroxy-5β-pregnan-20-one	79
11β,17α,20,21-Tetrahydroxypregn-4-en-3-one	*Clostridium paraputrificum*	5β-Pregnane-3α,11β,17α,20,21-pentol	79
3C=O → 3β-OH			
5α-Androstane-3,17-dione	*Caldariella acidophila*	(i) 3β-Hydroxy-5α-androstane-17-one	48
		(ii) 3β,17β-Dihydroxy-5α-androstane	
5α-Androstane-3,17-dione	*Rhodotorula mucilaginosa*	3β-Hydroxy-5α-androstan-17-one	75
17β-Hydroxy-5α-androstan-3-one	*Rhodotorula mucilaginosa*	5α-Androstane-3β,17β-diol	75
16β,17β-Epoxy-5α-androstan-3-one	*Curvularia clavata*	(i) 16β,17β-Epoxy-5α-androstane-3β,14-diol-7-one	29
		(ii) 16β,17β-Epoxy-5α-androstane-3β,5,7α-triol	
D-Homo-5α-androstane-3,6-dione	*Rhizopus nigrican*	3β,17α-Dihydroxy-D-homo-5α-androstan-6-one	30
D-Homo-5α-androstane-3,7-dione	*Rhizopus nigrican*	3β,17α-Dihydroxy-D-homo-5α-androstan-7-one	30
D-Homo-5α-androstane-3,17-dione	*Rhizopus nigrican*	3β,7β-Dihydroxy-D-homo-5α-androstan-17-one	30
11α-Hydroxy-D-homo-5α-androstan-3-one	*Rhizopus nigrican*	D-Homo-5α-androstane-3β,11α-diol	30
17α-Cyanomethylester-4-en-17β-ol-3-one	(i) *Rhodosporidium rubrum* or	17α-Cyanomethyl-5α-estrane-3β,17β-diol	77
	(ii) *Rhodotorula glutinis*		

Table 4 (*Continued*)

Substrate	Microorganism	Product	Reference
17α-Cyanomethylestra-4,9-dien-17β-ol-3-one	(i) *Rhodosporidium rubrum* or (ii) *Rhodotorula glutinis*	17α-Cyanomethylestra-4,9-dien-3,17β-diol	77
Pregn-4-ene-3,20-dione	*Aspergillus fumigatus*	3β-Hydroxy-5α-pregnan-20-one	33
Pregn-4-ene-3,6,20-trione	*Caldariella acidophila*	(i) 3β-Hydroxy-5α-pregnane-6,20-dione (ii) 3β-Hydroxypregn-4-ene-6,20-dione	48
5α-Pregnane-3,6,20-trione	*Caldariella acidophila*	3β-Hydroxy-5α-pregnane-6,20-dione	48
5α-Pregnane-3,11,20-trione	*Caldariella acidophila*	3β-Hydroxy-5α-pregnane-11,20-dione	48
7C=O → 7β-OH			
3α-Hydroxy-7-oxo-5β-cholanoic acid	*Clostridium absonum*	3α,7β-Dihydroxy-5β-cholanoic acid	52
17C=O → 17CH$_2$			
Androst-4-ene-3,17-dione	*Mucor* sp.	6,14α-Dihydroxyandrost-4-en-3-one	26
17C=O → 17α-OH			
3β-Hydroxy-D-homo-5α-androstan-17-one	*Rhizopus nigrican*	(i) D-Homo-5α-androstane-3β,17α-diol (ii) D-Homo-5α-androstane-3β,7β,17α-triol	30
17C=O → 17β-OH			
Androst-4-ene-3,17-dione	*Mycobacterium* NRRL B-12472	17β-Hydroxyandrost-4-en-3-one	80
	Mucor sp.	17β-Hydroxyandrost-4-en-3-one	26
	Aspergillus aureogulgens	17β-Hydroxyandrost-4-en-3-one	10
5α-Androstane-3,17-dione	*Caldariella acidophila*	5α-Androstane-3β,17β-diol	48
3β-Acetoxyandrost-5-en-17-one	*Streptomyces albus*	17β-Hydroxyandrost-4-en-3-one	81
3β-Hydroxy-D-homo-5α-androstan-17a-one	*Rhizopus nigrican*	D-Homo-5α-androstane-3β,17aβ-diol	30
20C=O → 20-OH			
Pregn-4-ene-3,20-dione	*Bifidobacterium adolescentis*	20-Hydroxypregn-4-en-3-one	79
3β-Acetoxypregn-5-en-20-one	*Streptomyces albus*	20α-Hydroxypregn-4-en-3-one	81
16α,17α-Epoxy-3β-hydroxy-5α-pregnan-20-one	*Arthrobacter simplex*	(i) 16α,17α-Epoxypregna-1,4-dien-3-on-20α-ol (ii) 16α,17α-Epoxypregn-4-en-3-on-20α-ol	63
21-Hydroxypregn-4-ene-3,20-dione	*Bifidobacterium adolescentis*	20,21-Dihydroxypregn-4-en-3-one	79
3α,21-Dihydroxy-5β-pregn-20-one	*Bifidobacterium adolescentis*	5β-Pregnane-3α,20,21-triol	79
11β,17α-Dihydroxypregn-4-ene-3,20-dione	*Bifidobacterium adolescentis*	11β,17α,20-Trihydroxypregn-4-en-3-one	79
3α,11β,17α-Trihydroxy-5β-pregnan-20-one	*Bifidobacterium adolescentis*	5β-Pregnane-3α,11β,17α,20-tetrol	79
11β,17α,21-Trihydroxypregn-4-ene-3,20-dione	*Bifidobacterium adolescentis*	11β,17α,20,21-Tetrahydroxypregn-4-en-3-one	79
3α,11β,17α,21-Tetrahydroxy-5β-pregnan-20-one	*Bifidobacterium adolescentis*	5β-Pregnane-3α,11β,17α,20,21-pentol	79

Table 5. Miscellaneous

Substrate	Microorganism	Product	Reference
Acetylation			
Cholesterol	*Arthrobacter* sp.	Cholest-5-en-3β-acetate	59
Sitosterol	*Anthrobacter* sp.	Stigmast-5-en-3β-acetate	59
Aromatization			
3β-Acetoxy-19-hydroxymethylandrost-5-en-17-one	*Corynebacterium simplex*	Estrone	82
3β-Hydroxy-19-hydroxymethylandrost-5-en-17-one	*Corynebacterium simplex*	Estrone	82
3α,12α-Dihydroxy-5β-cholanoic acid	*Pseudomonas* NCIB 10590	3-Hydroxy-9,10-secoandrosta-1,3,5(10)-triene-9,17-dione	11
Dehydration			
3α,6α-Dihydroxy-5β-cholanoic acid	*Pseudomonas* NCIB 10590	3-Oxochol-5-en-24-oic acid	49
3α,7α-Dihydroxy-5β-cholanoic acid	*Pseudomonas* NCIB 10590	(i) 3-Oxochola-4,6-dien-24-oic acid	51
		(ii) Pregna-4,6-dien-3-one-20-carboxylic acid	
		(iii) Androsta-4,6-diene-3,17-dione	
		(iv) Androsta-1,4,6-triene-3,17-dione	
		(v) 17β-Hydroxyandrosta-4,6-dien-3-one	
		(vi) 17β-Hydroxyandrosta-1,4,6-trien-3-one	
3α,7α,12α-Trihydroxy-5β-cholanoic acid	*Pseudomonas* NCIB 10590	(i) 12α-Hydroxyandrosta-4,6-diene-3,17-dione	55
		(ii) 3,12-Dioxochola-4,6-dien-24-oic acid	
		(iii) 12α-Hydroxypregna-4,6-dien-3-one-20-carboxylic acid	
		(iv) 12α-Hydroxychola-4,6-dien-3-on-24-oic acid	
Dehydroxylation			
3α,6α-Dihydroxy-5β-cholanoic acid	*Pseudomonas* NCIB 10590	(i) 3-Oxochol-4-en-24-oic acid	49
		(ii) 3-Oxochola-1,4-dien-24-oic acid	
		(iii) Androst-4-ene-3,17-dione	
		(iv) Androsta-1,4-diene-3,17-dione	
		(v) 17β-Hydroxyandrosta-1,4-dien-3-one	
3α,7α-Dihydroxy-5β-cholanoic acid	*Eubacterium* sp.	3α-Hydroxy-5β-cholanoic acid	52
3α,7β-Dihydroxy-5β-cholanoic acid	*Eubacterium* sp.	3α-Hydroxy-5β-cholanoic acid	52
3α,12α-Dihydroxy-5β-cholanoic acid	DSM-1444	(i) 12-Oxo-bisnor-5β-cholanoic acid	73
		(ii) 12α-Hydroxy-bisnor-5β-cholanoic acid	
3α,7α-12α-Trihydroxy-5β-cholanoic acid	*Pseudomonas* NCIB 10590	(i) 12α-Hydroxyandrosta-1,4-diene-3,17-dione	55
		(ii) 12α-Hydroxy-5β-androstane-3,17-dione	
		(iii) 3α,12α-Dihydroxy-5β-androstan-17-one	
		(iv) 5β-Androstane-3α,12α,17β-triol	
11β,17α,21-Trihydroxypregn-4-ene-3,20-dione	*Eubacterium lentum*	11β,17α-Dihydroxypregn-4-ene-3,20-dione	79
3α,11β,17α,21-Tetrahydroxy-5β-pregnan-20-one	*Eubacterium lentum*	3α,11β,17α-Trihydroxy-5β-pregnan-20-one	79
Hydrogenation			
17β-Hydroxyandrost-4-en-3-one	*Bacillus* sp.	17β-Hydroxy-5α-androstane-3,6-dione	39
Androst-4-ene-3,6,17-trione	*Caldariella acidophila*	5α-Androstane-3,6,17-trione	48

Table 5. (*Continued*)

Substrate	Microorganism	Product	Reference
6β-Hydroxyandrost-4-ene-3,17-dione	*Caldariella acidophila*	5α-Androstane-3,6,17-trione	48
Androst-4-ene-3,17-dione	*Aspergillus aureogulgens*	5β-Androstane-3,17-dione	10
17β-Hydroxyandrost-4-en-3-one	*Aspergillus aureogulgens*	5β-Androstane-3,17-dione	10
18-Hydroxyandrost-4-en-3-one-17β-carboxylic acid lactone	*Clostridium paraputrificum*	18-Hydroxy-5β-androstan-3-one-17β-carboxylic acid lactone	76
6β-Hydroxypregn-4-ene-3,20-dione	*Caldariella acidophila*	5α-Pregnane-3,6,20-trione	48
Pregn-4-ene-3,6,20-trione	*Caldariella acidophila*	5α-Pregnane-3,6,20-trione	48
21-Acetoxy-18-hydroxypregn-4-ene-3,20-dione	*Clostridium paraputrificum*	18,21-Dihydroxy-5β-pregnane-3,20-dione	76
Pregn-4-ene-3,20-dione	*Aspergillus aureogulgens*	(i) 5β-Androstane-3,17-dione (ii) 5β-Androstan-3α-ol-17-one	10
Pregn-4-ene-3,20-dione	*Bacillus* sp.	(i) 14α-Hydroxy-5α-pregnane-3,6,20-trione (ii) 11α-Hydroxy-5α-pregnane-3,6,20-trione	39
<u>Hydrolysis</u>			
3β-Acetoxyandrost-5-en-17-one	(i) *Penicillium oxalicum* FERM P-4729 or (ii) *Aspergillus terrens*	1α,3β-Dihydroxyandrost-5-en-17-one	27
3β-Acetoxyandrost-5-en-17-one	*Streptomyces albus*	3β-Hydroxyandrost-5-en-17-one	81
3β-Acetoxy-19-hydroxymethylandrost-5-en-17-one	*Corynebacterium simplex*	Estrone	82
17β-Acetoxyandrost-4-en-3-one	*Aspergillus aureogulgens*	17β-Hydroxyandrost-4-en-3-one	10
17β-Acetoxyandrost-4-en-3-one	*Streptomyces albus*	17β-Hydroxyandrost-4-en-3-one	81
3β-Acetoxypregn-5-en-20-one	*Streptomyces albus*	3β-Hydroxypregn-5-en-20-one	81
3β-Acetoxypregna-5,16-dien-20-one	*Streptomyces albus*	3β-Hydroxypregna-5,16-dien-20-one	81
21-Acetoxy-18-hydroxypregn-4-ene-3,20-dione	*Clostridium paraputrificum*	18,21-Dihydroxy-5β-pregnane-3,20-dione	76
3β,17α,21-Triacetoxypregn-4-en-20-one	*Flavobacterium dehydrogenans* and *Curvularia lunata*	17α-Acetoxy-11β,21-dihydroxypregn-4-ene-3,20-dione	23
3β,21-Diacetoxy-17-(methoxy methoxy) pregn-5-en-20-one	*Flavobacterium dehydrogenans* and *Curvularia lunata*	11β,21-Dihydroxy-17-(methoxy methoxy)pregn-4-ene-3,20-dione	21
21-Acetoxy-17α-hydroxy-16α-methyl pregna-4,9(11)-diene-3,20-dione	*Arthrobacter simplex*	17α,21-Dihydroxy-16α-methylpregna-1,4,9(11)-triene-3,20-dione	67
21-Acetoxy-3β,17α-dihydroxy-16β-methyl-5α-pregn-9(11)-ene-20-one	*Arthrobacter* 9-2	17α,21-Dihydroxy-16β-methylpregna-1,4,9(11)-triene-3,20-dione	68
<u>Hydrolysis cum oxidation</u>			
3β-Acetoxyandrost-5-en-17-one	*Streptomyces albus*	(i) Androst-4-ene-3,17-dione (ii) 17β-Hydroxyandrost-4-en-3-one	81
17β-Hydroxy-17α-methyl-5α-androstan-3β-acetate	*Nocardia* 76816 and *Arthrobacter* simplex A-1 (mixed culture)	17β-Hydroxy-17α-methylandrosta-1,4-dien-3-one	20
3β-Acetoxypregna-5,16-dien-3-one	*Streptomyces albus*	Pregna-4,16-diene-3,20-dione	81
3β-Acetoxypregn-5-en-20-one	*Streptomyces albus*	(i) Pregn-4-ene-3,20-dione (ii) 20α-Hydroxypregn-4-en-3-one	81
3β-Acetoxy-5α-pregn-16-en-20-one	*Arthrobacter* 9-2	Androsta-1,4-diene-3,17-dione	65
3β-Acetoxypregn-5-en-20-one	*Aspergillus aureogulgens*	(i) Androst-4-ene-3,17-dione (ii) 17β-Hydroxyandrost-4-en-3-one (iii) Testolactone	10

3β,21-Diacetoxy-17(methoxy methoxy) pregn-5-en-20-one	*Flavobacterium dehydrogenans* and *Curvularia lunata*	11β,21-Dihydroxy-17(methoxy methoxy) pregn-4-ene-3,20-dione	21
3β,17α,21-Triacetoxypregn-5-en-20-one	*Flavobacterium dehydrogenans* ATCC-13930	17α-Acetoxy-21-hydroxypregn-4-ene-3,20-dione	23
3β,17α,21-Triacetoxypregn-5-en-20-one	*Flavobacterium dehydrogenans* and *Curvularia lunata*	17α-Acetoxy-11β,21-dihydroxypregn-4-ene-3,20-dione	23
17β-Acetoxyandrost-4-en-3-one	*Aspergillus aureogulgens*	Androst-4-ene-3,17-dione	10
17β-Acetoxyandrost-4-en-3-one	*Streptomyces albus*	Androst-4-ene-3,17-dione	81
Oxidation			
17β-Hydroxyandrost-4-en-3-one	*Bacillus* sp.	17β-Hydroxy-5α-androstane-3,6-dione	39
16β,17β-Epoxy-5α-androstan-3-one	*Curvularia clavata*	16β,17β-Epoxy-3β,14-dihydroxy-5α-androstan-7-one	29
3β-Hydroxy-D-homo-5α-androstan-17a-one	*Rhizopus nigrican*	3β-Hydroxy-D-homo-5α-androstane-7,17a-dione	30
Pregn-4-ene-3,20-dione	*Bacillus* sp.	(i) 11α-Hydroxy-5α-pregnane-3,6,20-trione (ii) 14α-Hydroxy-5α-pregnane-3,6,20-trione	39
Androst-4-ene-3,17-dione	*Aspergillus aureogulgens*	Testolactone	10
17β-Hydroxyandrost-4-en-3-one	*Aspergillus aureogulgens*	Testolactone	10
17β-Acetoxyandrost-4-en-3-one	*Aspergillus aureogulgens*	Testolactone	10

a mixture of *Nocardia* 76816 and *Arthrobacter simplex* A-1 with an average yield of 71% [20]. 3β-21-Diacetoxy-17-(methoxy methoxy)pregn-5-en-20-one (**8**) on fermentation with *Flavobacterium dehydrogenans* and then with *Curvularia lunata* yielded 11β,21-dihydroxy-17-(methoxy methoxy)pregn-4-ene-3,20-dione (**9**) [21]. The effect of α-, β- and γ-cyclodextrin on the transformation of hydrocortisone to prednisolone by *Arthrobacter simplex* was examined [22]. It was observed that the cyclodextrin inhibited the precipitation of the substrate and the time required for the transformation could be lowered. Crystallographic monitoring of microbiological steroid transformations has been reported [23]. Product crystal formation was observed for the transformation of triolone triacetate (**10**) to Reichstein-S 17α-acetate (**11**) with *Flavobacterium dehydrogenans* ATCC 13930 at a concentration of 20 g/l. and for the conversion of hydrocortisone 17α-acetate (**12**) to prednisolone 17α-acetate with *Arthrobacter simplex* ATCC-6946 (2 g/l.). The compound **12** was obtained from **10** by a two-stage fermentation with *F. dehydrogenans* and *Curvularia lunata* NRRL 2380. At the concentration (1 g/l.) used for the second stage, crystal formation of **12** could not be observed. The progress of the first stage of the transformation could be studied by the powder diffraction technique.

NEWER MICROBIAL REACTIONS

9-Hydroxy-3-oxo-4,17(20)-pregnadiene-20-carboxylic acid (**13**), a novel transformation product was obtained in good yield from sitosterol by fermentation at 28° for 336 hr with *Mycobacterium fortuitum* in a medium (pH 7.0) containing (per litre) sitosterol (30 g), ucon (8 g), cerelose (5 g), NH_4Cl (3 g), $CaCO_3$ (3 g), trisodium citrate $2H_2O$ (3 g), Tween 80 (2 g), soybean meal (1 g), KH_2PO_4 (0.5 g) and urea (0.5 g) [24]. Moreover, 9-hydroxy-3-oxo-4,17(20)-pregnadiene-20-carboxaldehyde (**14**) was produced from sitosterol by a strain of *Mycobacterium fortuitum* NRRL B-12433 under the same fermentation conditions [25]. A Clemmensen type microbial reduction of the 17-keto group of androst-4-ene-3,17-dione by a *Mucor* sp. has been reported [26]. The major product obtained was characterized as compound **15**.

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 steroid dehydrogenations which have been demonstrated and the substrates have been classified alphabetically. Table 3 shows the side chain degradations achieved and reductions of keto groups are shown in Table 4. While in Table 3 the substrates have been arranged alphabetically, the substrates in Table 4 have been classified according to increasing order of the carbon atom bearing the ketone function which has been reduced. The miscellaneous reactions, e.g. acetylation, aromatization, dehydration, dehydroxylation, hydrogenation, hydrolysis, hydrolysis cum oxidation and oxidation are given in Table 5.

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