FACULTY OF ENGINEERING & TECHNOLOGY
DEPARTMENT OF BIOTECHNOLOGY

STEROID BIOTRANSFORMATION

Introduction

•Naturally occurring steroids possess remarkable hormonal properties which are of therapeutic importance to human well-being, such as hormones of adrenal cortex (cortisone, cortisol, corticosterone)

•The progestational hormone (progesterone), the androgens or male sex hormones(testosterone, dihydrotestosterone) and the estrogens or female sex hormones (estradiol, estrone, etc.)

•Steroids are small organic molecules with a characteristic molecular structure containing four rings of carbon atoms synthesized in steroidogenic tissues

•It include many hormones, alkaloids, and vitamins

•It act on target sites to regulate a cascade of physiological functions

The pharmaceutical industry has great interest in the biotransformation of steroids for the production of steroid hormones.

• Steroid hormones and their derivatives have been used for a wide range of therapeutic purposes.

• Beside the established utilization as immunosuppressive, anti-inflammatory, anti-rheumatic, progestational, diuretic, sedative, anabolic and contraceptive agents, recent applications of steroid compounds include the treatment of some forms of cancer, osteoporosis, HIV infections and treatment of declared AIDS.

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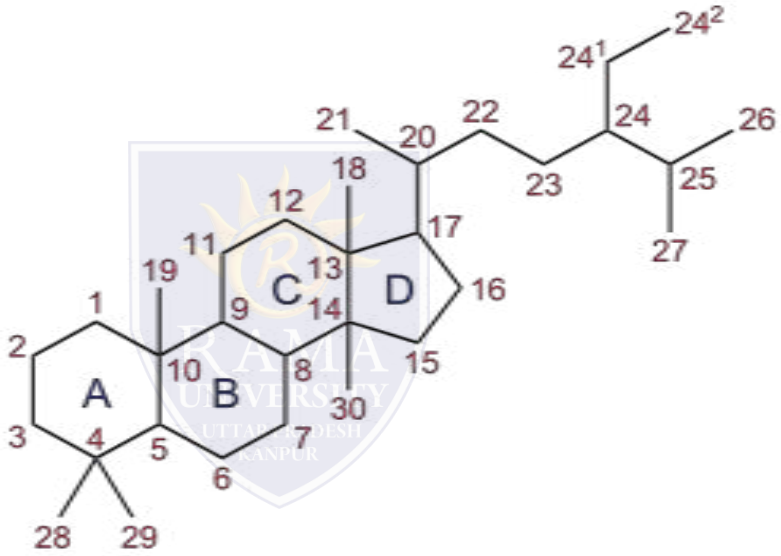
Structure of Steroid



Transformation of Steroids

- Transformation of steroids means conversion of precursor steroids to important drug intermediates and further conversion of these intermediates to active compounds by simple chemical or microbial processes.
- The chemical synthesis and transformations of steroids requires multiple steps and makes the use of reagents that have health risks and cause serious environmental disposal problems.
- Alternative is microbial transformation

Microbial transformation

- These involves simple, chemically defined reactions catalyzed by enzymes present in the cell. Microbial cells provide the enzymes to catalyze the transformation reactions

- The microorganisms have got the ability to chemically modify a wide variety of organic compounds. These microbes during the bioconversion provide enzymes which act upon and convert the organic compound into other compounds or modify it.

- Microbial transformations cleave the complex side chains of precursor steroids in one single step and incorporate desirable modifications in steroid nucleus.

- Microbial transformations are regiospecific and stereospecific, whereby organic compounds are modified into desirable isomers of products involving simple chemically defined reactions catalyzed by the enzymes in the microbial cells.

Types of Steroid transformation

Oxidation

- Hydroxylation
- Dehydrogenation
- Epoxidations
- Oxidation to ketone through hydroxylation
- Ring AAromatization
- Degradation of steroid nucleus

Transformation Steroid

Oxidation

Oxidation of alcohols to ketone: 3β-OH to 3-keto

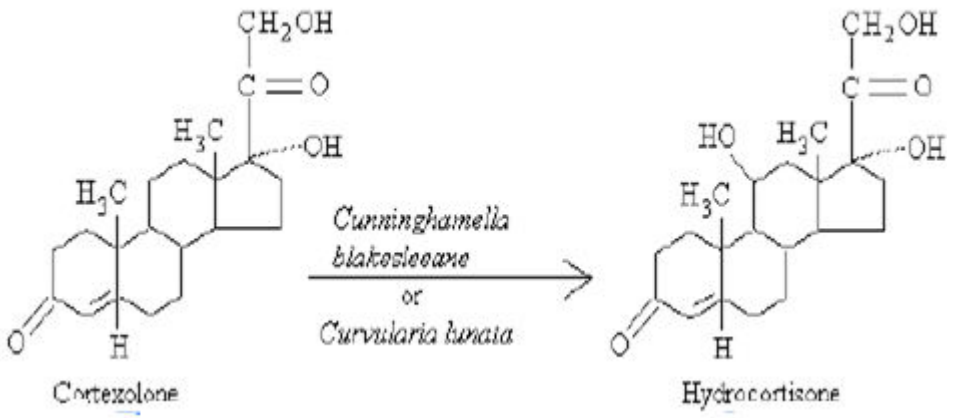
Side chain cleavage of steroids

Decarboxylation of acids

Hydroxylation

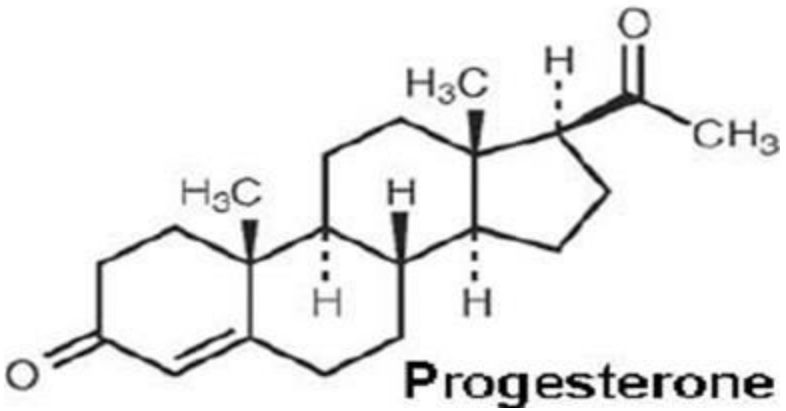
•Hydroxylation involves the substitution of hydroxyl group directly for the hydrogen at the position, be it α or β, in the steroid with a retention of configuration.

•The oxygen atom in the hydroxyl group is derived form molecular oxygen (gaseous), not from water, and the hydroxyl group thus formed always retains the stereochemical configuration of the hydrogen atom that has been replaced.



•Fungi are the most active hydroxylating microorganisms, but some bacteria particularly the *Bacilli, Nocardia and Streptomyces* show fair good activity.

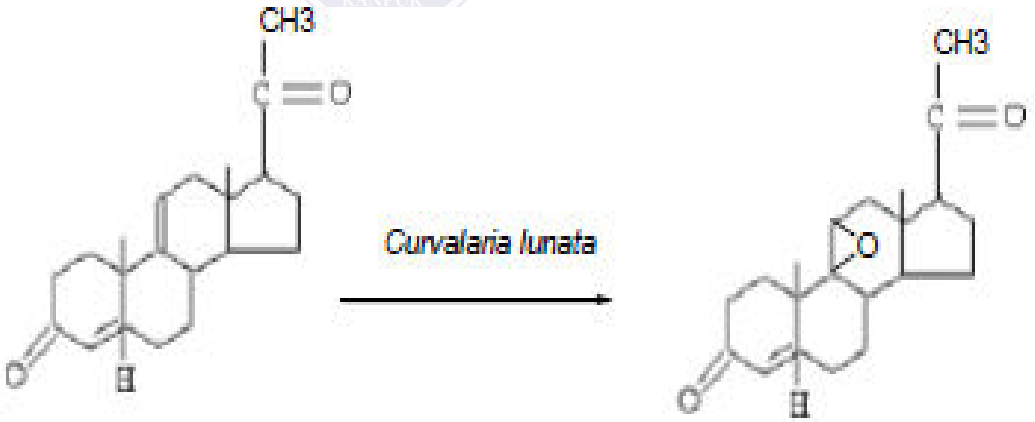
•The hydroxylation at the 11-position of progesterone was one of the first hydroxylation described



Epoxidation

•The epoxidation of steroidal double bonds is a rare example of biological epoxidation.

•The 9,11- epoxidation of 9(11)-dehydro-compounds , and the 14, 15-epoxidation of 14(15)-dehydrocompounds ,using *Curvalaria lunata.*



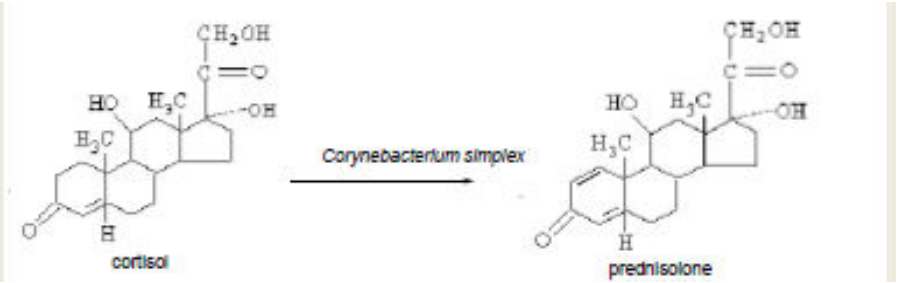
Dehydrogenation

• Dehydrogenation with the concomitant introduction of a double bond has been reported for all four rings of the steroid nucleus

• The introduction of unsaturated bonds in Ring A is the only reactions of commercial importance.

Example :

• In 1955, Charney and co-worker observed that they could greatly enhance the anti-inflammatory properties of cortisol by causing the compound to be dehydrogenated at 1st position by *Corynebacterium simplex. The* resultant product, prednisolone, was 3-5 times more active than the parent compound and produced fewer side effects.



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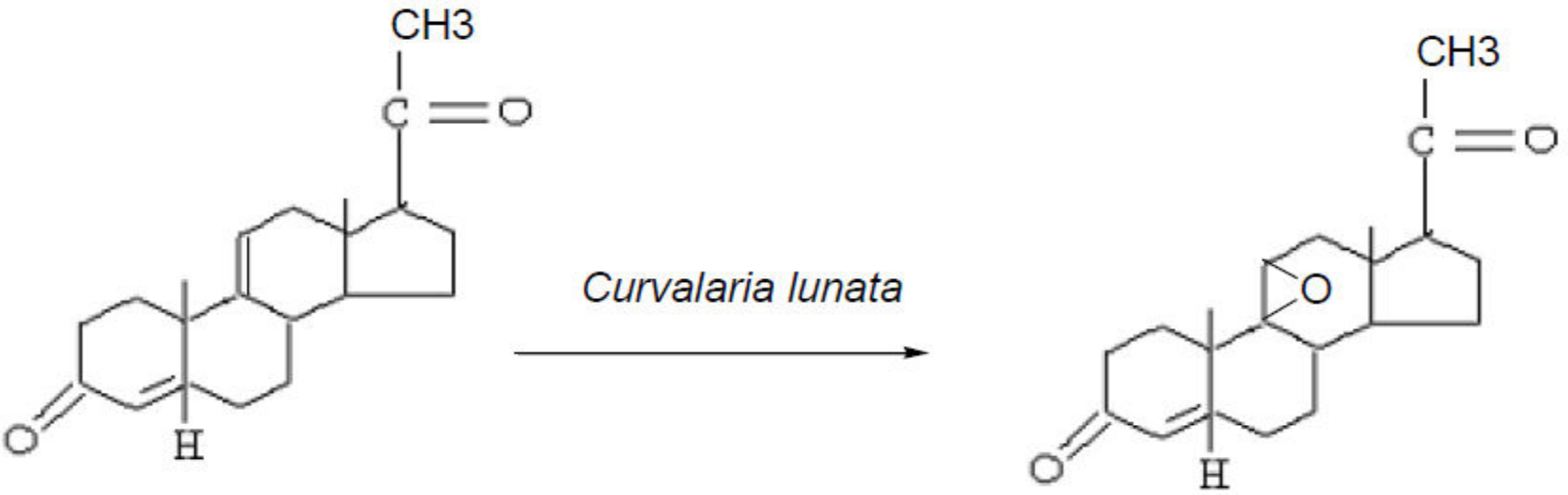

Ring A Aromatization

•The microbial aromatization of suitable steroid substrates can lead to ring A aromatic compounds, particularly the estrogens which constitutes an important ingredient in oral contraceptives drugs and play important role in replacement therapy for menopause treatment

•Cell free extracts of *Pseudomonas testosteroni* could transform 19- nor testosterone into estrone with small quantities of estradiol-17β.

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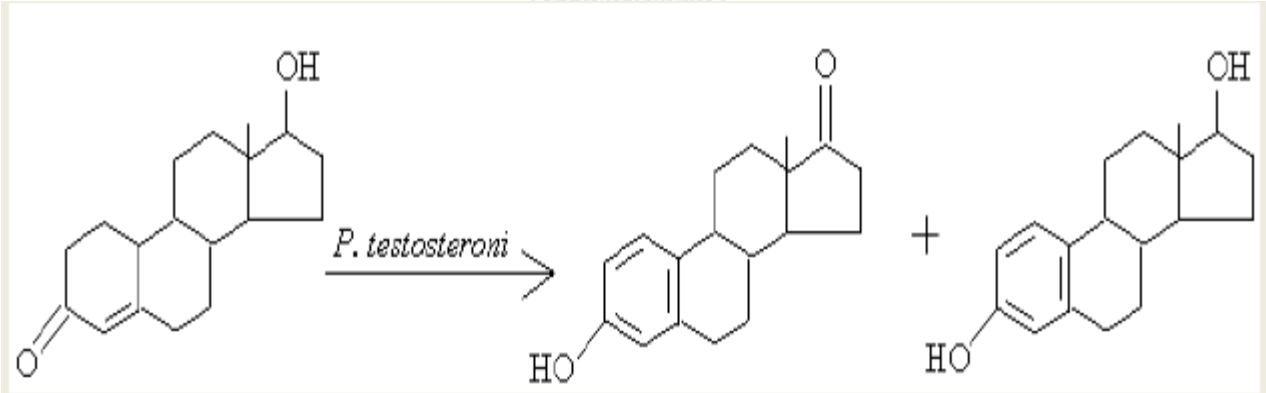
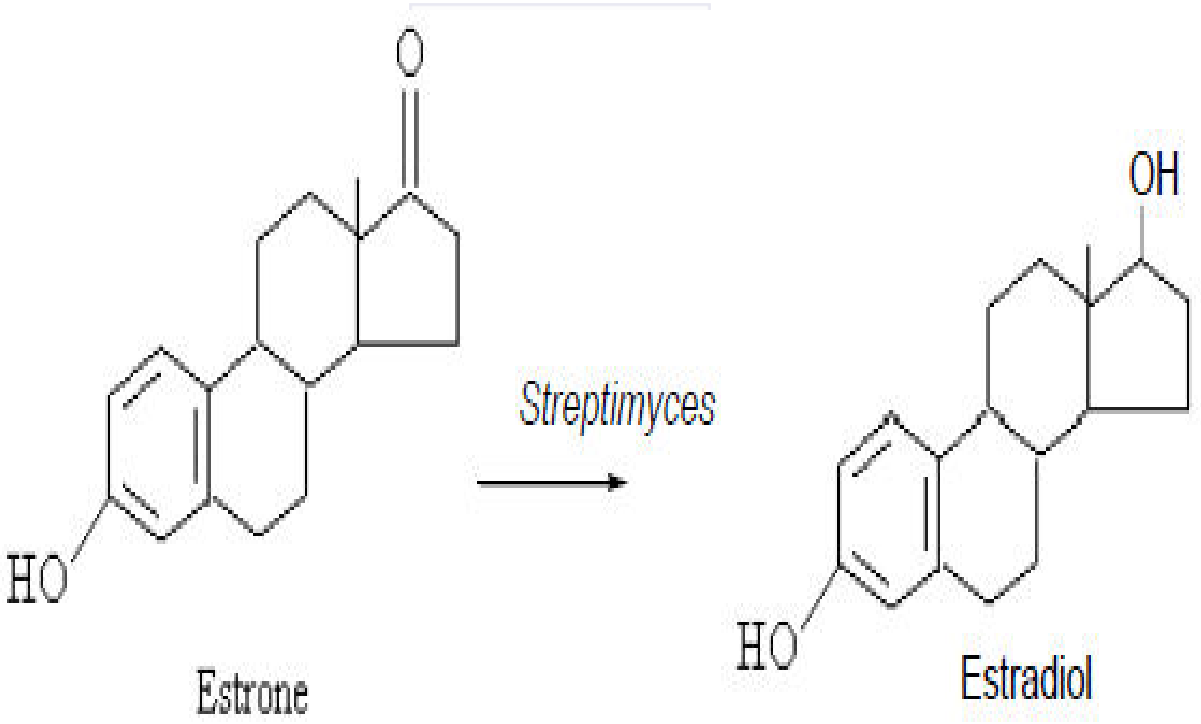


19-nortestosterone Estrone Estradoil-17β

Reduction

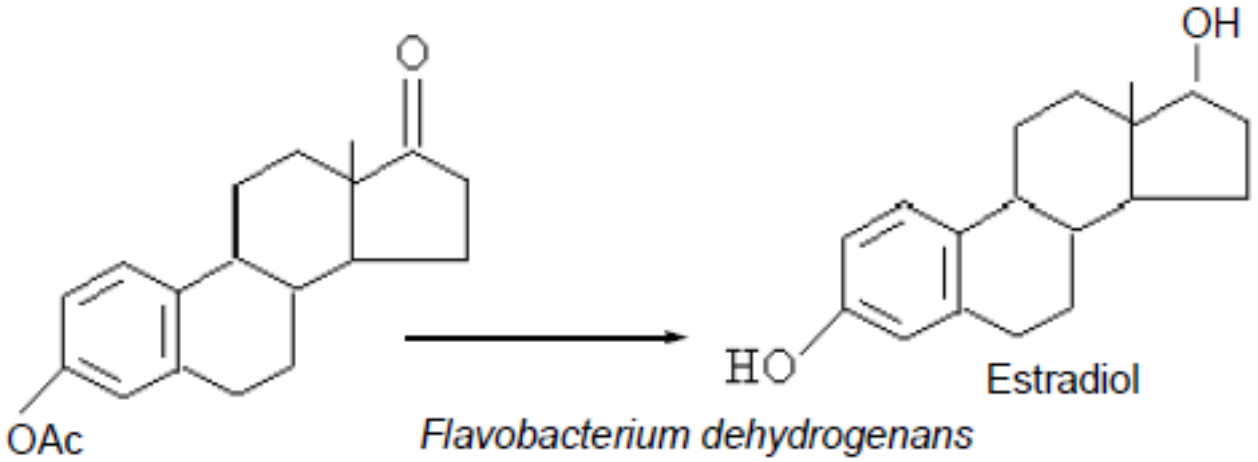
Reduction of aldehydes and ketones to alcohols



Hydrolysis

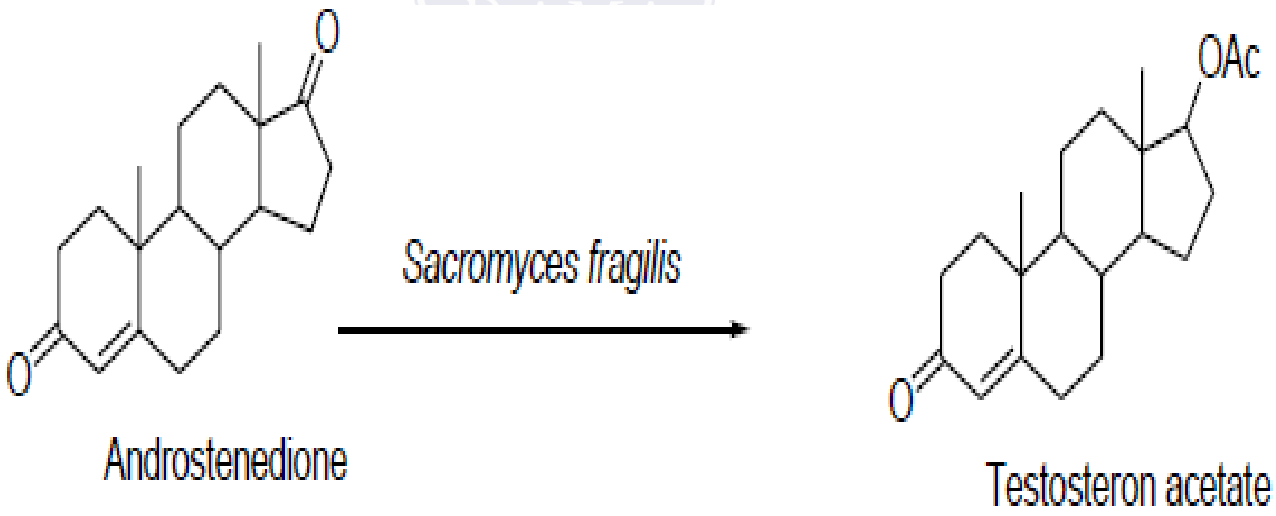
Hydrolysis of esters

Flavobacterium dehydrogenans contain a specific enzyme acetolase which hydrolyses the steroidal acetates

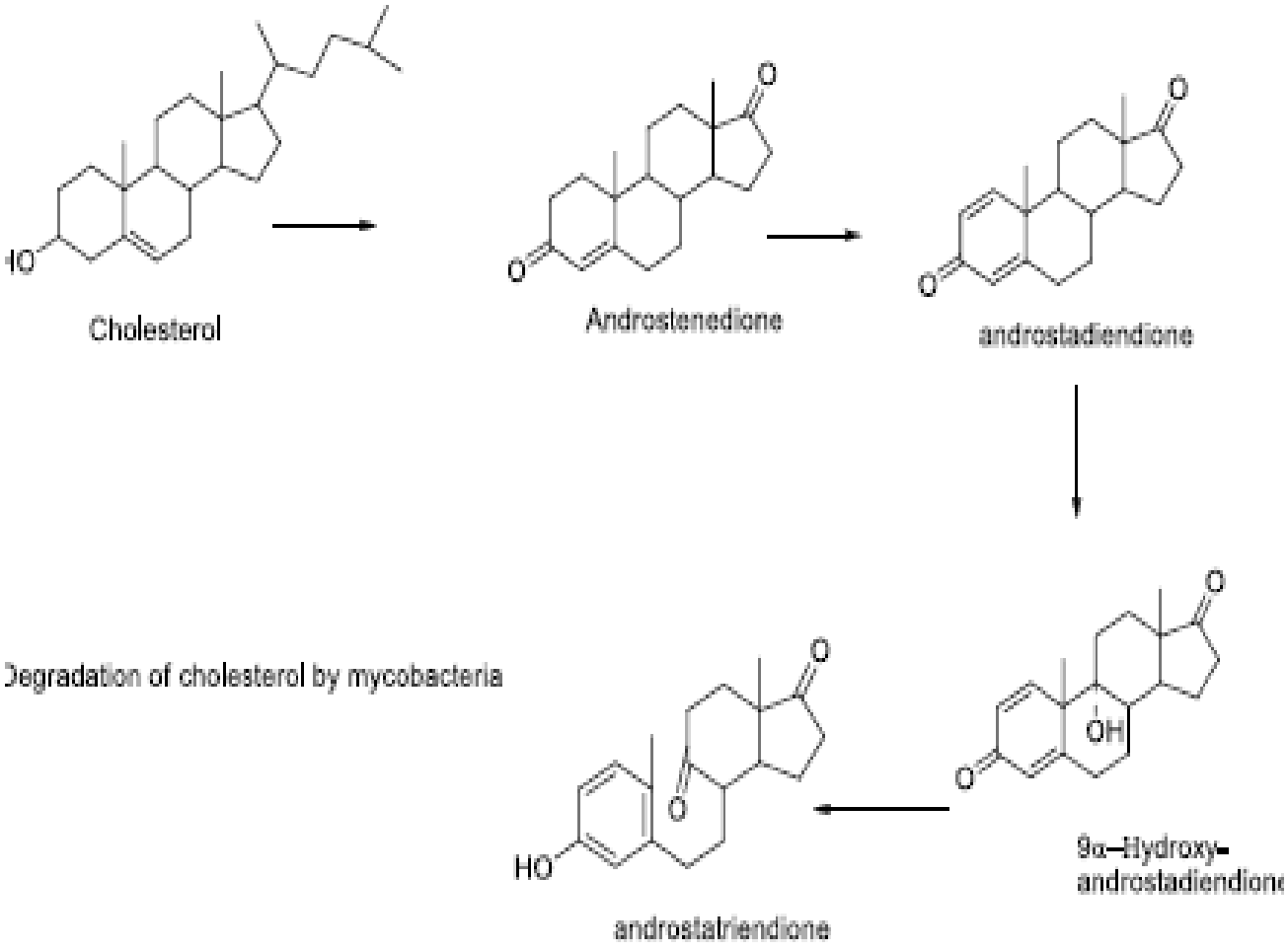


Esterification

Usually involve acetylation



Steroid Ring Degradation



Degradation of cholesterol by mycobacteria

Fermentation condition of some steroids

M/O	Steroid substrate	Steroid product	Length of incubation , temperature, aeration
Alcaligenes faecalis	Cholic acid	Ketocholic acids (90-100%)	2 days (monoketo acid) 4 days (diketo acid) 6 days (triketo acid) 37-39 °, surface culture
Fusarium solani	Progesterone	1,4-androstadiene-3, 17-dione(85%)	4 days , 25 °C , rotary shaker (100 rpm)
Corynebacterium mediolanum	21-acetoxy -3 β-hydroxy -5-pregnen-20-one	21-hydroxy-4-pregnene-3, 20-dione (30%)	6 days , 36-37 °C , pure oxygen with agitation

Advantages

•Microorganisms have great potential for inducing new or novel enzyme systems capable of converting foreign substrates.
•Microorganisms are capable of producing unique enzymes which are stable toward heat, alkali and acid.
•A combination of microbial transformation and chemical transformations (chemo-enzymatic synthesis) can be exploited for partial, as well as the total synthesis of the organic compounds

Disadvantages

• If the substrate is toxic, it can kill the microorganisms. Hence no transformation will be observed.

• Very low chemical yields are obtained due to the involvement of a complex biological system