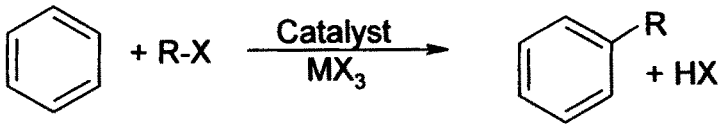
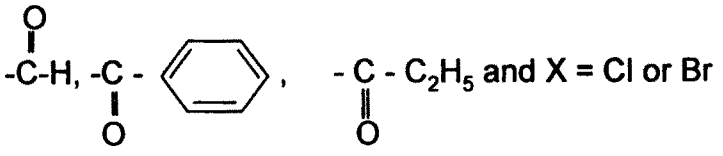
Chart - 1

Scheme - 1

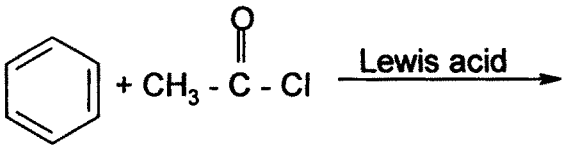


(1) **(2)** **(3)**

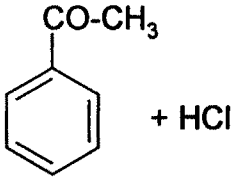
Where R = CH_3, C_2H_5, $CH(CH_3)_2$, $C(CH_3)_3$, $-\overset{O}{\overset{\|}{C}}-CH_3$



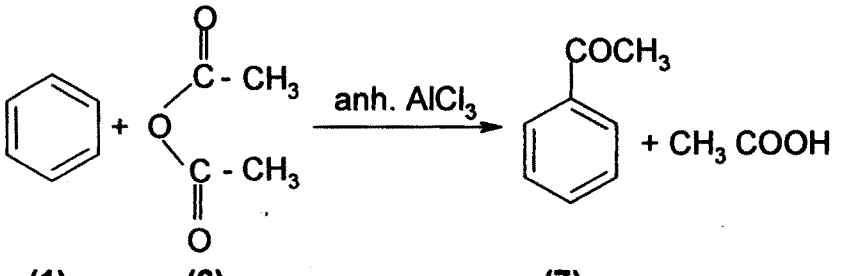
Scheme - 2



(1) **(4)**



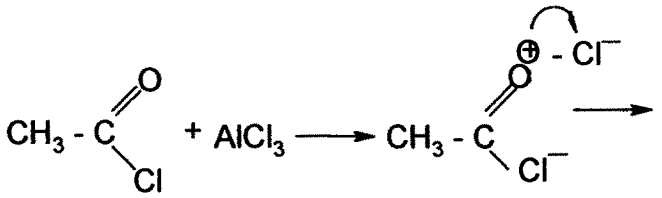
(5)



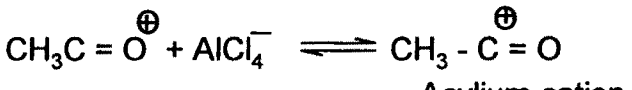
(1) **(6)** **(7)**

CHART - 2

Scheme - 3

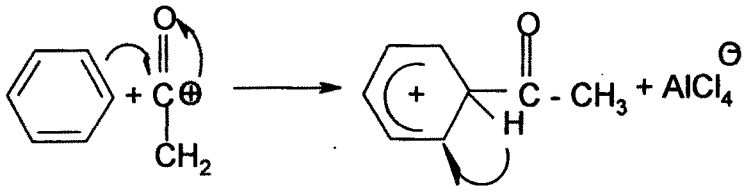


(4)

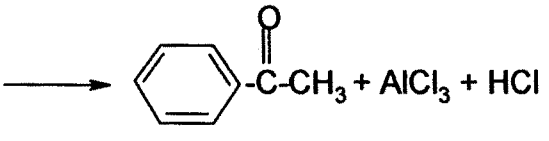


Acylium cation

(8)



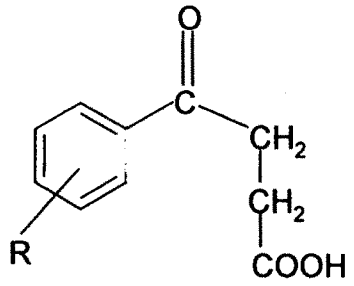
(9) **(10)**



(11)

should be chart-3

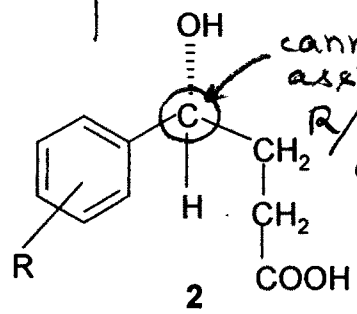
CHART - 1 — chart NO repeated



1

1a. R = H,
1b R = -CH_3,
1c R = -OCH_3

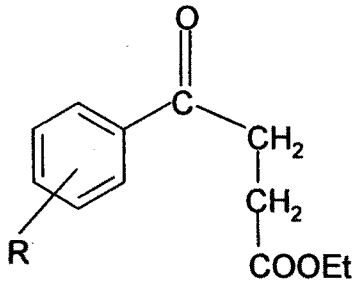
R. arrhizus



cannot assign R/S config.

2

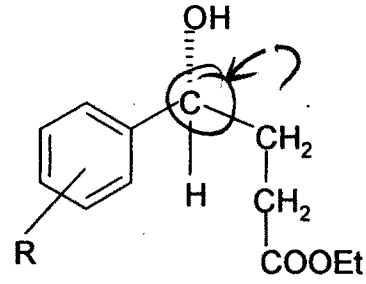
2a. R = H,
2b R = -CH_3,
2c R = -OCH_3



3

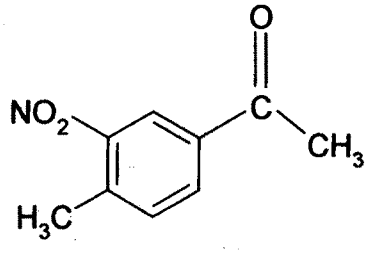
3a. R = H,
3b R = -CH_3,
3c R = -OCH_3

R. arrhizus



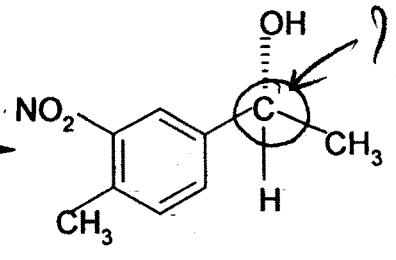
4

4a. R = H,
4b. R = -CH_3
4c. R = -OCH_3



5

R. arrhizus



6

CHAPTER - I

A BRIEF REVIEW ON ENZYME CATALYSED REACTIONS

CHAPTER – 1

A BRIEF REVIEW ON ENZYME CATALYSED REACTIONS

INTRODUCTION

In human life the optically active compounds play a versatile role as they are the essential components of life itself, proteins, carbohydrates and DNA. Now-a-days importance of using bioactive substances in optically pure form is increasing tremendously. More and more drugs, pesticides etc are being marketed as pure optical isomers. Due to this there is increasing demand for efficient methods for their industrial production.

The first antique example of microbial transformation is the fermentation of sugar for the preparation of alcohol. The foundation of biotechnology was laid with the classical experiments of L. Pasteur, who observed the formation of glycerol as a minor component of sugar fermentation. Another important compound viz. citric acid has also been obtained by the fermentation process. The original strategy involved the degradation of sucrose by the micro organism. Today the same methodology is followed with minor modifications.

Microorganisms have the ability to chemically modify a wide variety of organic compounds and these changes are called biological or microbial transformations or more generally bio-conversions. In these enzymatic reactions, the substrate may be metabolized, but in some cases the conversions may take place without energy gain (cometabolism).

Bioconversions are more preferable due to the following reasons.

Substrate specificity – Only one specific reaction step is normally catalysed by an enzyme.

Site specificity (rigiospecificity) – If several functional groups of one type are present in the molecule, only one specific position may be affected.

Stereo selectivity – If a racemic mixture is used as a starting material, only one specific enantiomer is converted. If a center of asymmetry appears as a result of the enzyme reaction, the reaction product is normally optically active.

Reaction conditions – Enzymatic reaction do not cause destruction of sensitive substrate, due to mild conditions of conversion. Several reactions can be combined either in one fermentation step using an organism with suitable enzyme systems or by stepwise conversions using different micro organisms. The reactions cause less environmental hazard as they take place chiefly in water.

BIOCATALYSTS :

Most of the enzymes are sensitive. Under certain conditions, enzymes can be remarkably stable. Some of them can tolerate hostile environments such as temprature greater then 100°C and pressure beyond 200 bar [1]. Enzymes can be reused if they are immobilized.

Many enzymes are capable of accepting synthetic substrate of an unrelated structural type and can convert them, often exhibiting the same high specificities as far the natural counterparts. It is a general trend that the more complex the enzyme's mechanism the narrower the limit for the acceptability of "foreign" substrates.

It is generally true that an enzyme displays it's highest catalytic power in water, which in turn represents something of a nightmare for the organic chemist if it is the solvent of choice.

Although the activity is usually lower in such an environment, many other advantages can be accrued thus making many processes more effective by using biocatalysts in organic solvents.

ADVANTAGES OF BIOCATALYSTS

1) Effective catalyst

Enzymes are very effective catalysts and when we used enzyme in the chemical processes generally the rates are increased, compared to those of the corresponding non enzymatic reactions by a factor of $10^8 - 10^{10}$. It is possible to increase the rate of reaction upto 10^{12} [2]. Enzymes exhibit a high substrate tolerance by accepting a large variety of man made synthetic substances and often they are not required to work in water. The aqueous medium can sometimes be replaced by an organic solvent.

Like all catalysts, enzymes only accelerate a reaction but they have no impact on the position of the thermodynamic equilibrium of the reaction. Some enzyme catalyzed reactions can be run in both directions.

Some major exceptions, where equivalent reaction types cannot be found in nature are the Diel's – Alder reaction [3] and the cope rearrangement although (1,1) sigmatropic rearrangements such as the claisen – rearrangement are known. [4].

2) Catalysts working in mild conditions

Enzymes act in a pH range of about 5-8, typically around 7 and in a temp. range of 20-40°C, preferably at around 30°C. This minimizes problems of undesired side reactions such as decomposition, isomerisation, racemization and rearrangement.

3) Ecofriendly catalyst

Biocatalysts are completely degraded in the environment.

4) Selectivity of Enzyme Catalyst

Chemoselectivity

Since the purpose of an enzyme is to act on a single type of functional group, other sensitivity functionalities, which would normally react to a certain extent under chemical catalysis survive. e.g.- enzymatic ester hydrolysis does not show any propensity for acetal cleavage.

Regioselectivity and diastereoselectivity

Due to their complex, three dimensional structure enzymes may distinguish between functional groups which are chemically situated in different regions of the same substrate molecules [5].

Enantioselectivity

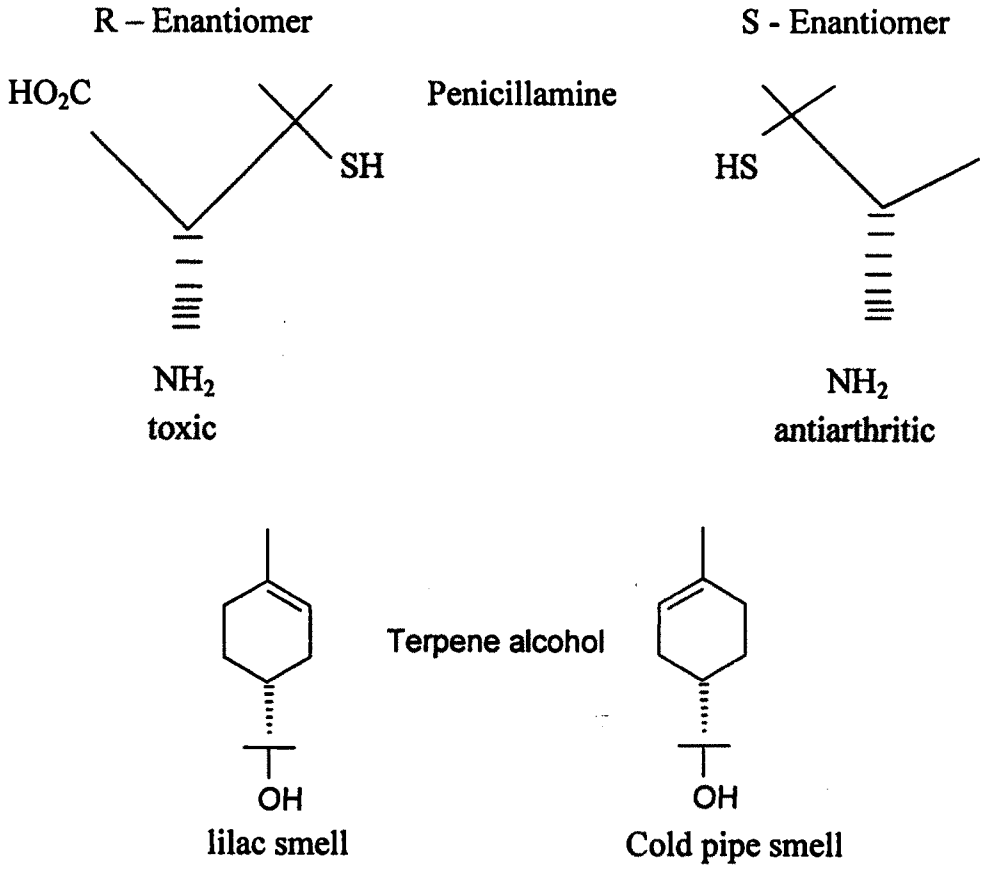
Almost all enzymes are made from L-amino acids and thus are chiral catalysts [6]. As a consequence, any type of chirality present in the substrate molecule is recongnized upon the formation of the enzyme substrate complex. So a prochiral substrate may be transformed into an optically active product and both enantiomers of a racemic substrate may react at different rates affording a kinetic solution.

These latter properties collectively constitute the 'specificity' of an enzyme and represent it's most important feature for selective and asymmetric exploitation [7]. This key feature was recognized by E. Fischer in the year 1898 [8].

All the major biochemical events taking place in an organism are governed by enzymes. Since the majority of them are highly selective with respect to the chirality of a substrate, it is obvious that the enantiomers of a

given bioactive compound such as a pharmaceutical or an agrochemical cause different biological effect [9]. So they must ~~be~~ differ as two different species. The isomer with the highest activity is denoted as the 'eutomer' where as it's enantiomeric counterpart possessing less or even undesired activities is termed as the 'distomer'. The ratio of the activities of both enantiomers is defined as the 'endismic ratio'. The various examples of different biological effects are listed below

Biological effects of enantiomers



The principle of asymmetric synthesis [10] makes use of enantiomerically pure auxillary reagents which are used in catalytic or sometimes in stoichiometric amounts. They are often expensive and cannot be recovered in many cases.

DISADVANTAGES OF BIOCATALYSTS

1) Structure of enzyme

Enzymes are provided by nature in only one enantiomeric form. Since there is no general way of creating mirror image enzymes (from D amino acids). It is impossible to invert the chiral induction of a given enzymatic reactions by choosing the other enantiomer of the biocatalysts, a strategy which is possible if chiral chemical catalysts are involved.

2) Parameters of enzymatic reactions

Enzymes requires narrow operation parameters. Mild reaction conditions can sometimes turn into a drawback. If a reaction proceeds only slowly under given parameters of temp or pH, there is only a narrow scope for alteration. ~~If we~~ increased in temp., and/or pH concentration may lead to deactivation of the protein. The narrow temp. range for the operation of enzymes prevents radical changes, ~~have been reported~~ [11].

3) Solvent for enzyme

Enzyme display their highest catalytic activity in water. Due to its high boiling point and high heat of vapourisation water is usually the least desired solvent of choice for most organic reactions. Furthermore, the majority of organic compounds are only poorly soluble in aqueous media.

Thus shifting enzymatic reactions from an aqueous to an organic medium would be highly desired but the unavoidable price one has to pay is usually some loss of activity [12].

4) Care of enzyme

Enzyme may cause allergies to human beings. However, this may be minimized if enzymes are regarded as chemicals and handled with the same care while working in enzymatic reactions.

PROPERTIES OF ENZYME

The polyamide chain of an enzyme is kept in a three dimensional structure, the one with the lowest Δ G [13].

This is believed to be determined by its primary sequence and is called the 'primary structure.' For an organic chemist it may be compared with a ball of yarn.

Due to the natural aqueous environment, the hydrophilic polar groups such as – COOH, -OH, $-NH_2$, -SH, $-CONH_2$ are mainly located on the outer surface of the enzyme with the lipophilic substitutents – the aryl and alkyl chains buried inside. The surface of an enzyme is covered by a layer of water, which is tightly bound and generally cannot be removed by lyophilization.

This residual water, accounting for about 5-10% of the total dry weight of a freeze-dried enzyme is called the 'structural water' [14]. It is a distinctive part of the enzyme necessary to retain the enzyme's three dimensional structure and it differs significantly in its physical state from the 'bulk water' of the surrounding solution. There is very restricted rotation of the 'bound water' and it cannot freely reorient upon freezing. Exhaustive drying of an enzyme (e.g. by chemical means) would force the molecule to change its conformation resulting in a loss of activity. The whole structure is stabilized by a large number of relatively weak bonding forces such as Van der – Waal's interactions of aliphatic chains, π-π stacking of aromatic units or salt bridges between charged

part of the molecule. The only covalent bonds, besides the main polyamide backbone are S-S disulfide bridges.

Consequently enzymes are intrinsically unstable in solution and can be deactivated by denaturation, caused by increased temp., an extrime pH or an unfavourable dielectric environment such as high salt concentration.

The types of reaction leading to an enzyme deactivation are listed below [15].

1) Dehydration of peptide chains starts at around 40-50°C. Most of these are reversible and so relatively harmless.
2) Hydrolysis of peptide bonds in the backbone in particular at asparagines units, occurs at more elevated temps.
3) Thiol groups may interchange the S-S disulfide bridges, leading to a modification of covalent bonds within the enzyme.
4) Elimination and oxidation reaction cause the final destruction of the protein.

The three dimensional structure of such enzymes is often the same as those derived from meso-philics [16] but generally they possess less asparagines residues and more salt on disulfide bridges. More recently genetic engineering has become a powerful tool to obtain stable mutant enzymes.

CLASSIFICATION OF ENZYMES

At present almost 3000 enzymes have been recognized by the international Union of Biochemistry [17] and if the speculation that there are still about 25000 enzymes existing in nature is true [18] and till 90% of these are unknown to us. Therefore only a minor fraction of the enzymes are already investigated (roughly 300, ~15% is commercially available.

10%

300 is 10% of 3,000 & not 15%

For its identification every enzyme has got a 4 digit number Ec A.B.C.D. where Ec stands for Enzyme Commission with the following properties encoded.

A = Main type of reaction

B = Subtype indicates the substrate type or type of transferred molecule

C = Nature of the co-substrate

D = Individual enzyme number

As shown in table no. 1 enzymes have been classified into six categories according to the type of reaction they can catalyze.

The importance of practical application for organic synthesis is not at all evenly distributed among the different enzyme classes as may be seen from the initially column shown below. Further more due to the wide spread use of crude enzyme preparations often one does not know which enzyme is actually responsible for the biotransformation.

Table - 1

Classification of enzymes

Enzymes class	Reaction type
1. Oxidoreductases	$Oxid^n$-Red^n: oxyenation C-H, C-C, C=C bonds, or overall removal add^n of H atom equivalent
2. Transferases	Transfer of groups: aldehydic, ketonic acyl, sugar, phosphoryl or methyl
3. Hydrolases	Hydrolysis formation of esters, amides, lactones, lactams, epoxides, nitriles, anhydrides, glycosides
4. Lyases	Addition-elimination of small molecules on C=C, C=N, C=O bonds
5. Isomerases	Isomerisation such as racemization, epimerization
6. Ligases	Formation cleavage of C-O, C-S, C-N, C-C bonds con comitant triphosphate cleavage

Catalytic activities are measured in several different systems. The standard unit system is the International unit (1 I.U.) = 1 μ mol of substrate transformed per min. but other units such as μ mol/min or n mol/hour are also common. Another system of units is based on the katal (1 kat = 1 mol s^{-1} of substrate transformed) but it has not yet been widely accepted. The activities which are observed when synthetic organic compounds are used as substrate are

often significantly below the values which could be expected with natural substrate.

The efficiency of microbial transformations (where the catalytic activity of enzymes involved cannot be measured) is characterised by a so called 'productivity number' (PN) [19] defined as

$$PN = n\ prod / m\ dry.t$$

Where n prod = amount of product

m dry = amount of dry cell mass

t = time of transformation

This number resemble specific activity as defined for pure enzymes but includes also other several important factors such as inhibition, transport phenomena and concentration. A high PN for a given transformation indicates an efficient process giving a high output / unit volume and time.

SOURCES OF ENZYMES OR ENZYME SOURCES

The majority of enzymes used for biotransformation in organic chemistry are employed in a crude form and are relatively inexpensive. The preparations typically contain only about 1-30% of actual enzyme the remainder being inactive proteins, stabilizers, buffer salts or carbohydrates from the fermentation broth from which they have been isolated. It must be noted that, crude preparations are often more stable than purified enzymes.

The main sources for enzymes are as follows [20].

1) The detergent industry produces many proteases in huge amounts. These are largely used as additives for detergents to effect the hydrolysis of proteinogenic impurities.

2) The food industry uses proteases for meat and cheese processing and numerous lipases for the amelioration of fats and oils [21].

3) Numerous enzymes can be isolated from slaughter waste or cheap mammalian organs such as kidney or liver. Alternatively, microbial sources can be utilized. A small fraction of enzymes is obtained from plant sources.

4) Pure enzymes are usually very expensive and thus are mostly sold by the unit, whole crude preparations are often shipped by the kg. Since the techniques for protein purification are becoming easier, thus making their isolation more economically feasible, the use of pure enzymes in biotransformations is steadily increasing.

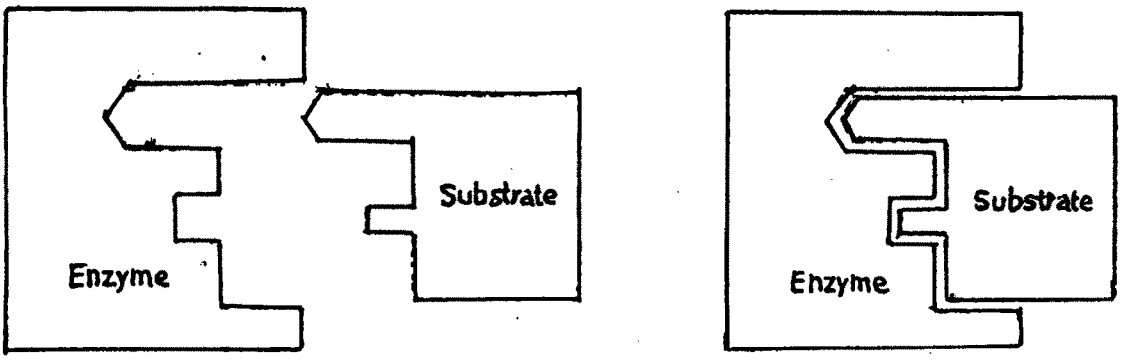
MECHANISM OF ENZYME CATALYSED REACTION

Lock and Key Mechanism

The first proposal for a general mechanism of enzymatic action was developed by E. Fischer in 1894 [22].

It assumes that an enzyme and its substrate mechanistically interact like a lock and a key, respectively. Although this assumption was quite sophisticated at that time, it assumes a completely rigid enzyme structure.

Schematic representation of the lock and key mechanism.



According to Fischer small substrates should be transformed at higher rates than larger substrates because the access to the active site would be easier.

Induced – Fit Mechanism

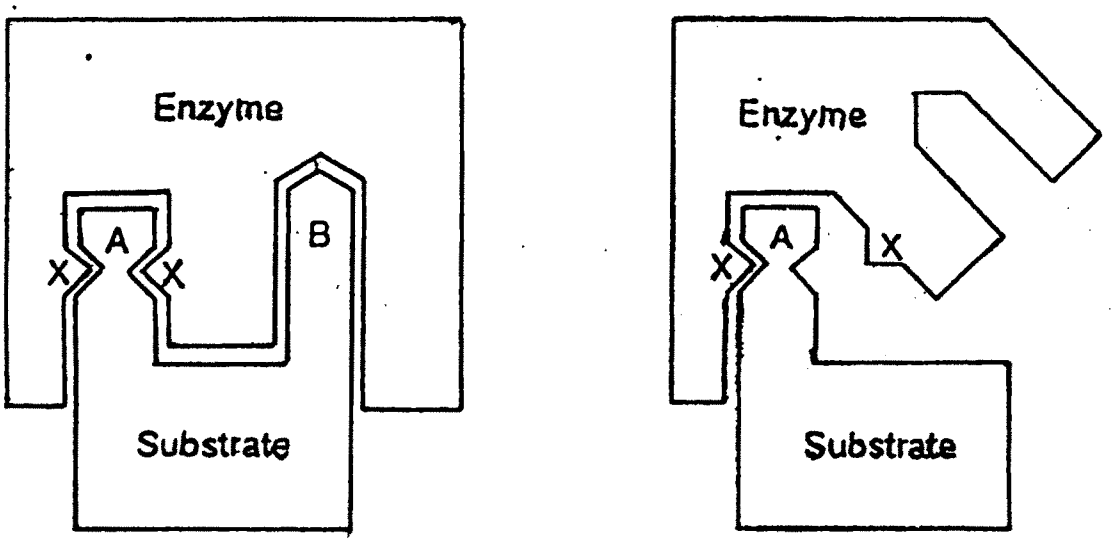
According to Induced – Fit Mechanism enzymes are not entirely rigid. This statement is made by Koshland Jr. in the year 1961.

It assumes that upon approach of a substrate during the formation of the enzyme substrate complex the enzyme can change its conformation under the influence of the substrate structure so as to wrap around its guest.

A similar picture is given by the interaction of a hand (the substrate) and a glove (the enzyme). This model can indeed explain why in many cases several structural features on a substrate are required.

These structural features may be located quite a distance from the actual site of the reaction e.g. lipases.

Schematic representation of the Induced-Fit mechanism



Here A = reactive group of substrate
X = complementary reactive groups of enzyme (the chemical operator)
B = substrate

Substrate part B forces the enzyme to adopt a different (active) conformation. Both of the active group X of the enzyme the 'chemical operators' are then positioned in the right way to effect catalysis.

If part B is missing no conformational change (the induced fit) takes place and thus the chemical operators stay in their inactive state.

Desolvation and Solvation Substitution Theory

According to M.J.S. Dewar [24] it assumes that the kinetic of enzyme reactions have much in common with those of gas-phase reactions. If a substrate enters the active site of the enzyme, it replaces all of the water molecules from the active site of the enzyme.

Then a formal gas phase reaction can take place which mimics two reaction partners interacting without 'disturbing' solvent.

In solution the water molecules impede the approach of the partners so the reaction rate is reduced.

This 'desolvation' theory has recently been substituted by a 'solvation-substitution theory' [25].

It is based on the assumption that the enzyme would not be able to strip off the water which is surrounding the substrate to effect a 'desolvation' because this would be energetically unfavoured.

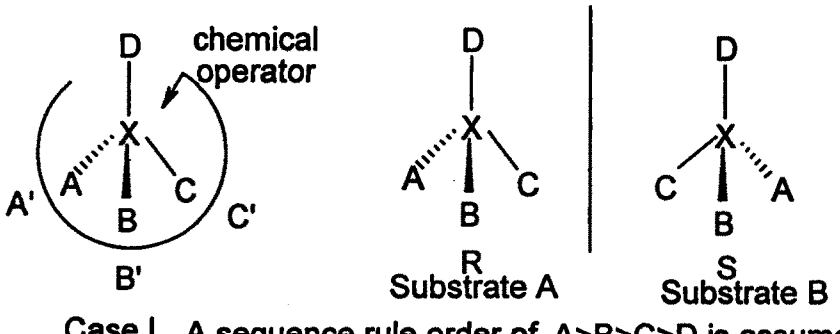
Instead the solvent is displaced by another polar environment by a so called 'solvation substitution'. Thus the hydrophobic substrates are replacing the water in the hydrophobic site of the enzyme [26].

THREE POINT ATTACHMENT RULE

This theory is putforth by A.G. Ogston [27]. It is assumed that, to get high degree of enantio-selection, a substrate must be held firmly in three dimensional space. So there must be at least three different points of attachment of the substrate onto the active site [28]

SCHEMATIC REPRESENTATION OF ENZYMATIC ENANTIOMER DISCRIMINATION

Chart No. 1



Case I A sequence rule order of A>B>C>D is assumed

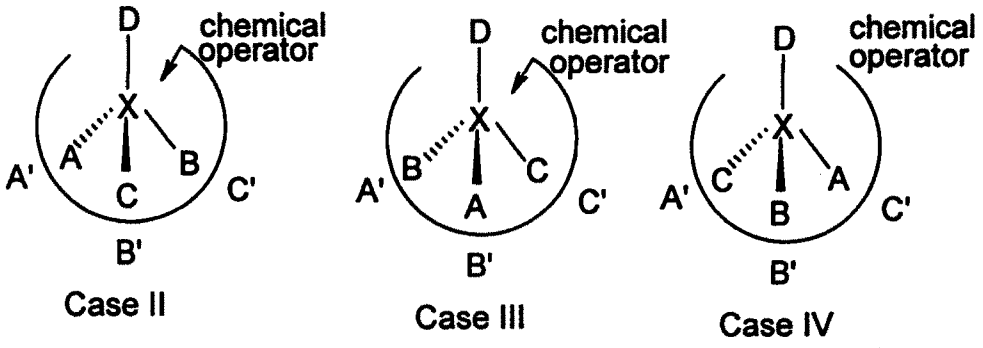


Chart No. 1

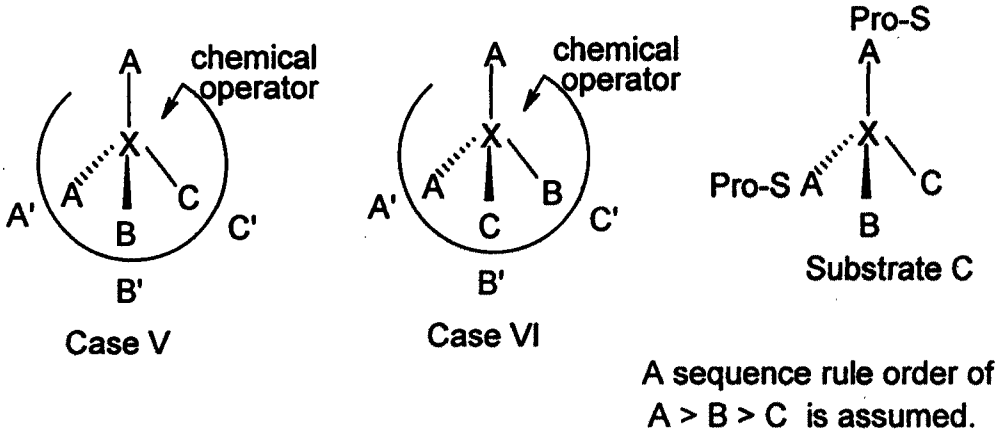
I) Enantiomer A is a good substrate by allowing optimal interaction of its groups (A,B,C) with their complementary binding site areas of the enzyme (A',B',C').

It ensures an optical orientation of the reactive groups (D) towards the chemical operator which is required for a successful transformation.

II) – Through IV :- Enantiomer B is poor substrate because optimal binding and orientation of the reactive group D is not possible. Thus poor catalyst will be observed.

If a prochiral substrate (c) bearing two chemically identical but stereochemically different enantiotopic group (A) is involved the same model can be applied to rationalize the favored transformation of one of the two leading to an enantiotopos differentiation.

Schematic representation of enzymatic enantiotopos discrimination



Case V- shows good binding of a prochiral substrate C to the complementary enzymes binding sites with the pro-(R) group out of the 2 reactive groups being positioned to the chemical operator.

Case VI – positioning of the pro-(s) reactive group towards the chemical operator results in poor orientation of the other functions to their complementary sites resulting in poor catalysis.

As a consequence the pro (R) group is cleaved preferentially to its pro (S) counterpart.

Generally many functional groups and sometimes also co-ordinated metal ions have to work together in the active site of the enzyme to effect catalysis. Individual enzyme mechanism have been elucidated in certain cases where the exact three dimensional structure is known.

For most of the enzymes used for the biotransformation of synthetic organic compounds, assumptions are made about their molecular action.

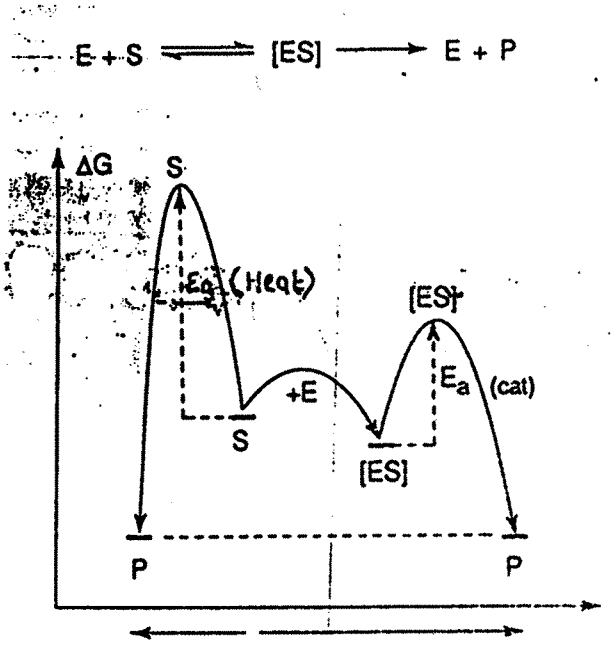
⟶ Kinetic study of enzymatic reason

In every catalytic reaction, an enzyme (E) accelerates the reaction by lowering the energy barrier between substrate (S) and product (P) the activation energy (Ea).

$$E + S \longrightarrow [ES] \longrightarrow E + P$$

The origin of this catalytic power the rate acceleration has generally been attributed to transition state stabilisation of the reaction by the enzyme [29], assuming that the catalyst binds more strongly to the transition state than to the ground state of the substrate by a factor approximately equal to the rate acceleration [30].

Energy diagram of catalysed versus uncatalyzed reaction



Uncatalysed enzyme cataslyed

Reaction co-ordinate

S – substrate, P – product, E – Enzyme

ES – enzyme substrate, complex, ≠, denotes a transition state

Ea – activation energy

The various reactions that can be catalysed by microorganisms are as follows.

Oxidation – Hydroxylation, epoxidation, dehydrogentaion oxidation of alcohols and aldehydes. Oxidative degradation of alkyl, carboxyalkyl or oxoalkyl chains, oxidative removal of substituents, oxidative deamination, oxidation of heterofunctions and oxidative ring fission.

Reduction - Reduction of organic acids, aldehydes, ketones and hydrogenation of C=C bonds, reduction of heterofunctions, dehydroxylation and reductive elimination of substituents.

Hydrolysis - Hydrolysis of esters amides, lactones, ethers, lactams etc., Hydration of C=C bonds and epoxides.

Condensation - Dehydration, O-and N-acylation, glycosidation, esterification, lactonisation and amination.

Isomenrisation - Migration of double bonds of oxygen functions, racemisation and rearrangements.

Thus various types of bioconversion reaction have been utilized in preparations of drugs, agrochemicals, pheromones, natural products, steroids. In addition to these applications microbial transformations of alkanes, alicyclic, aromatic and heterocyclic compounds, of terpene and of alkaloids have been described.

ENZYME CATALYSED REDUCTION REACTIONS

The different types of enzyme are widely used in redox reactions [31]. These enzymes are divided into three classes.

1) dehydroxygenases
2) oxygenases
3) oxidases

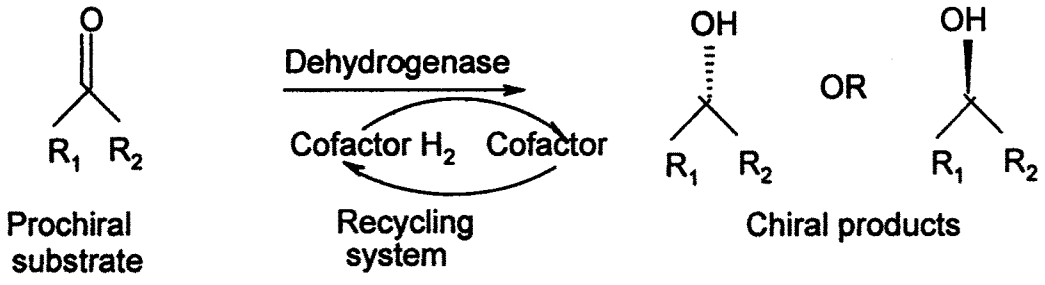
The former group of enzymes has been used for the reduction of carbonyl groups of aldehydes or ketones and of C=C bonds. The substitutions pattern of the substrate also affect the course of reaction. The reverse process (alcohol oxidation) is not so importance because of destruction of chiral center (1,1).

In contrast oxygenases – named for using molecular oxygen as a cosubstrate have been shown to be particularly useful for oxidation reactions. They are able to catalyze the functionalisation of non activated C-H or C=C bonds, affording a hydroxylation or epoxidation procedure respectively.

CHART - 1

Scheme-1 (1,1)

Reduction reactions catalyzed by dehydrogenases



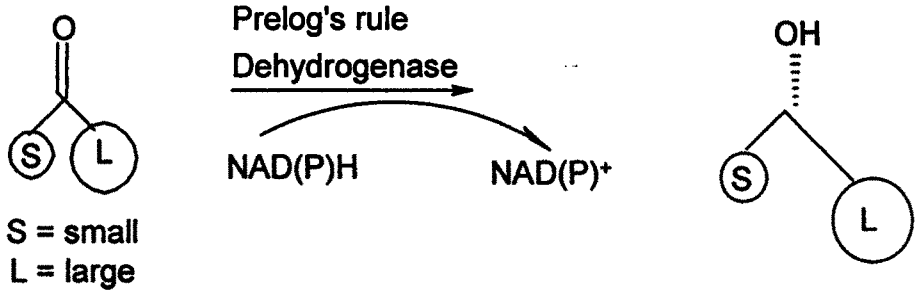
Prochiral substrate

Recycling system

Chiral products

Scheme - (1, 2)

→ **Prelog's role for the asymmetric reduction of ketones**



S = small
L = large

APPLICATIONS OF ISOLATED ENZYMES

Various ketones can be reduced stereoselectivley using dehydrogenases to give chiral secondary alcohols [32]. In this reaction, the enzyme delivers the hydride preferentially either from the Si - or the re-side of the ketone to give, for simple systems, the (R) or (S) alcohols resp. For most cases, the stereochemcial course of the reaction, which is mainly dependent on the steric requirements of the substrate it is shown by simple model is known as 'Prelog's rule' [33] (1,2).

Some of the commercially available dehydrogenases are as follows.

1) yeast alcohol dehydrogenase (YADH)
2) horse liver alcohol dehydrogenase (HLADH)
3) thermoanaerobium brochii alcohol dehydrogenase (TBADH)

All these obeys 'Prelog's rule. (1,2) Microbial dehydrogenases which lead to the formation of (R) alcohols are known, but they are not commercially available.

1) Yeast – ADH has a very narrow substrate specificity and in general, only accepts aldehydes and methyl ketones [34]. As a consequence, cyclic ketones and those with carbon chains larger than a methyl group are excluded as substrates.

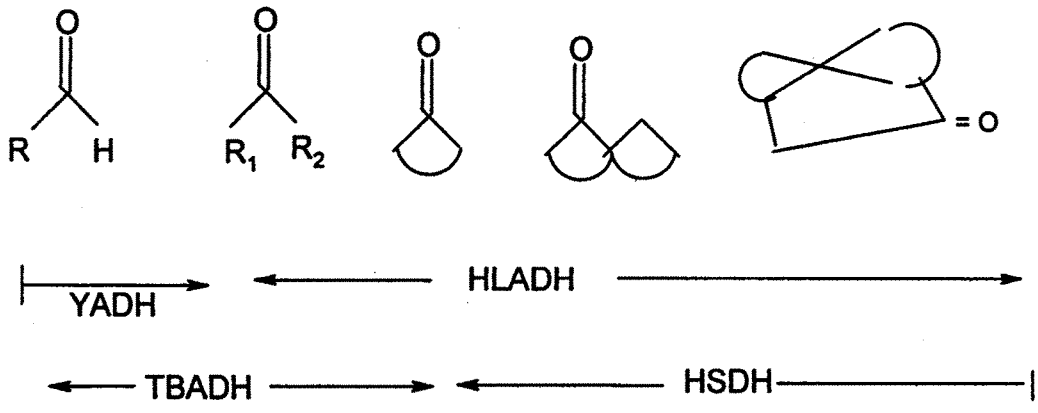
So YADH is only of limited use for the preparation of chiral secondary alcohols. Some of the commonly used dehydrogenases are shown in fig, with reference to their preferred size of their substrates [35] (2,1).

2) Horse liver ADH is a very catholic enzyme with a broad substrate specificity and a narrow stereospecificity so it is most widely used dehydrogenase in

CHART - 2

Scheme - (2,1)

Preferred substrate size for dehydrogenases



Scheme (2,2)

Distereoselective reduction of alkylthiopyranones using HLADH

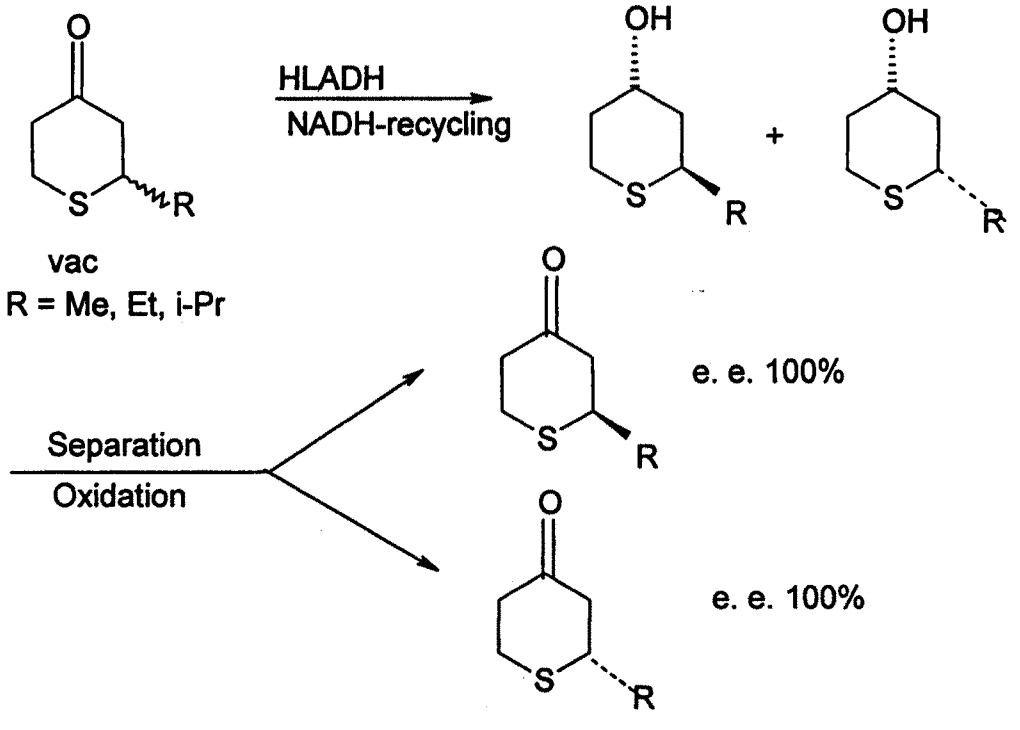
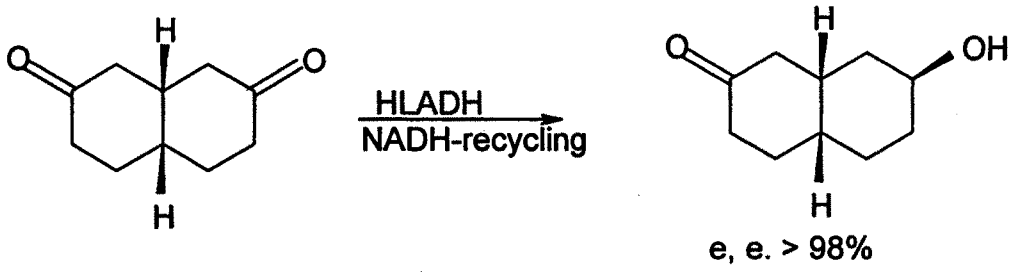


CHART - 3

Scheme (3,1)

Asymmetrization of prochiral diketones using HLADH

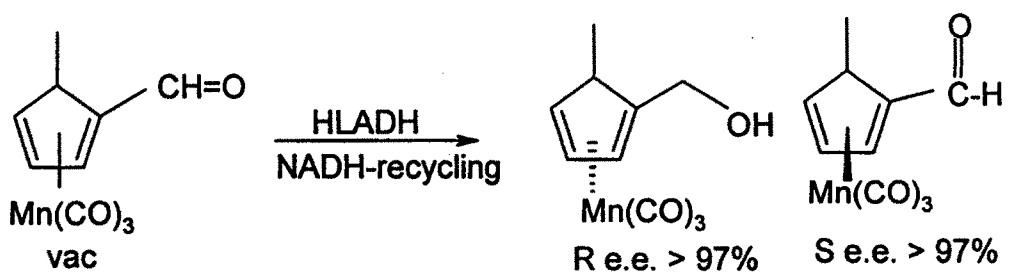


e, e. > 98%

e, e. > 98%

Scheme (3,2)

Reduction of an organometallic aldehyde using HLADH



vac

R e.e. > 97%

S e.e. > 97%

biotransformations [36]. The most useful applications are found in the reduction of medium ring monocyclic ketones and bicyclic ketones [37].

Monocyclic and bicyclic racemic ketones have been resolved using HLADH with fair to excellent specificities [38]. Even sterically demanding cage shaped polycyclic ketones were readily accepted [39].

The HLADH catalyzed reduction of 2-alkyl thio pyran-4-ones [40] (2,2) did not stop at 50% conversion but proceeded in a distereoselective fashion to give a mixture of cis and trans –(S) – alcohols.

Asymmetrization of prochiral diketones using HLADH (3,1) is also repeated in literature. Both the cis and trans forms of the decalinediones (3,1) were reduced to give (S) alcohols with excellent optical purity. Also the reduction of an organometallic aldehyde using HLADH have been reported (3,2). The wide substrate tolerance of HLADH is demonstrated by the resolution of organometallic derivatives possessing axial chirality [41].

YADH and HLADH are not useful for the asymmetric reduction of open chain ketones. Recently isolated alcohol dehydrogenase from the bacterium Thermoanaerobium brochii (TBADH) has been used for this type (4,1) conversions. TBADH is remarkably thermostable (upto 85°C), also it can tolerate the presence of organic solvents. Open chain methyl and ethyl ketones are readily reduced by TBADH to the corresponding secondary alcohols generally with excellent specificities [42]. In general TBADH obeys prelog's rule. The majority of synthetically useful ketones can be transformed into the corresponding chiral secondary alcohols by choosing the appropriate enzyme. α keto acids may be transformed into either (R) or (S)-2- hydroxy acids using NADH dependent lactate dehydrogenases (LDH) of different origins.

REDUCTION OF ALDEHYDE AND KETOINE USING WHOLE CELL

Whole microbial cell contain multiple dehydrogenases which are able to accept synthetic substrates, all the necessary cofactors and the metabolic pathways for their regeneration. All the enzymes and cofactors are well protected within their natural cellular environment.

The productivity of microbial conversions is usually low since the majority of non-natural substrates are toxic to living organisms and are therefore only tolerated at low concentrations (~0.1 to 0.3%). Different strains of a micro-organisms may possess different specificities thus it is important to use exactly the same culture to obtain comparable results [43].

Low stereoselectivities may cause following reasons –

A substrate may be reduced by a single oxidoreductase with different transition states for the two enantiomers or the enantiotopic faces.

If the two enzymes, each with high but opposite stereochemical preference complete for the same substrate, the relative rates of the individual reactions, the optical purity of the product depends on the substrate concentration [44].

Some general technique can be applied to enhance the selectivity of microbial reduction reactions :

- Substrate modification e.g. by variation of protecting groups which can be removed after the transformation.
- Variation of the metabolic parameters by immobilization.
- Variation of the fermentation conditions .
- Sceening of microorganisms to obtain strains with the optimum properties.

- Selective inhabition of one of the competing enzymes.

REDUCTION OF ALDEHYDE AND KETONES BY BAKER'S YEAST

Baker's Yeast (saecharomyces cerevisiae) is by far the most widely used microorganism for the asymmetric reduction of ketones [45 & 46]. It is ideal for nonmicrobiologists, because it is readily available. Its use does not require sterile fermenters and therefore can be handled using standard laboratory equipment. The first comprehensive review [47] was published in the year 1949.

Ketones

→ Simple aliphatic and aromatic latones are reduced by fermenting yeast according to Prelog's rule to give the corresponding (S) – alcohols in good optical purities. Long chain ketones such as n-propyl-n-butyl ketone and several bulky phenyl ketones are not accepted. One long alkyl chain is tolerated if the other moiety is the methyl group.

REDUCTION OF ALIPHATIC AND AROMATIC KETONES USING BAKER'S YEAST (4,2)

A wide range of functional groups can be tolerated adjacent to the ketone including chloro, bromo, perfluoroalkyl, nitro, hydroxyl, dithioanyl and even

→ Silyl and germyl-groups [48].

small 'f'

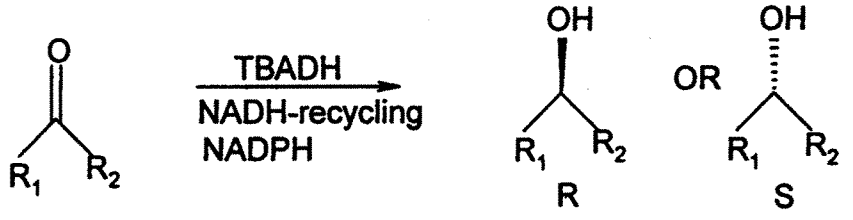
REDUCTION OF ACYCLIC β-KETOESTERS USING BAKER'S YEAST (4,3)

Acidic β-ketoesters are readily reduced by yeast to yield β-hydroxy esters [49] which serve as chiral starting materials for the synthesis of β-lactams insect pheromones and carotneoids [50]. It is obvious that the enantio selectivity and the stereochemical preference of the β-hydroxy ester depends on the relative size of the alkoxy moiety and the ω-substitutent of the ketone with

CHART - 4

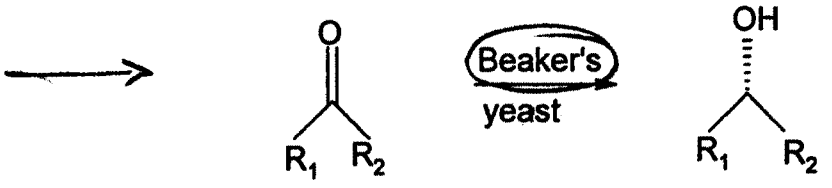
Scheme (4,1)

Enantioselective reduction of ketones using TBADH



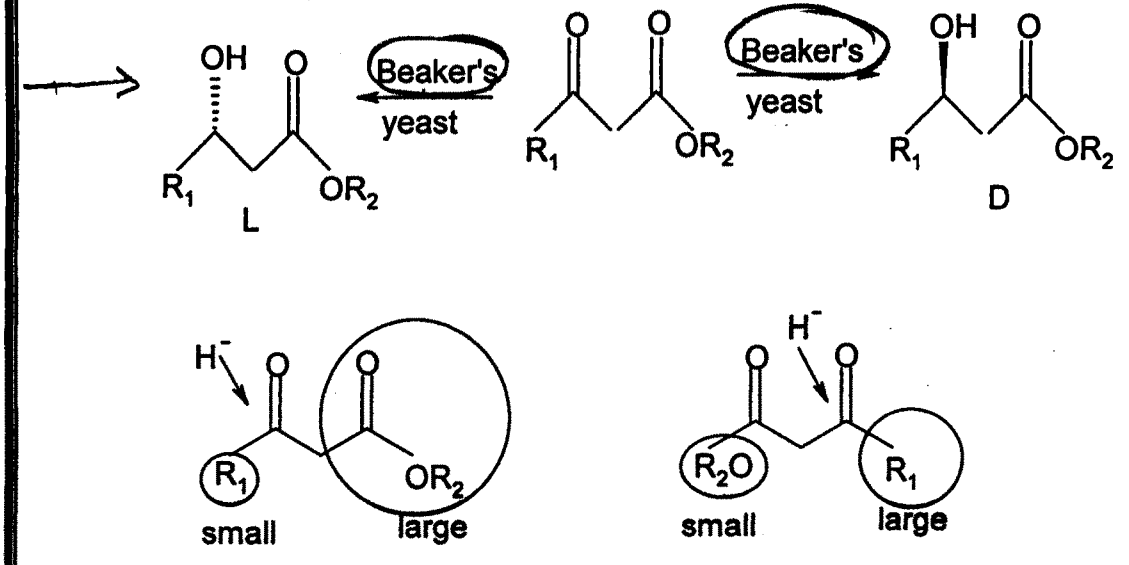
Scheme (4,2)

Reduction of aliphatic and aromatic ketones using Baker's yeast



Scheme (4,3)

Reduction of acyclic β ketoesters using Baker's yeast



the nucleophilic attack of the hydride occurring according to Prelog's rule as shown in above reaction.

As a consequence, the absolute configuration of the alcoholic center may be directed by substrate modification using either the corresponding short or long chain alkyl ester. The reason for this divergent behaviour is not due to an alternative fit of the substrate in a single enzyme but rather to the presence of a number of different dehydrognenses, possessing opposite stereochemical preferences, which complete for the substrate [51].

A D-specific enzyme belonging to the fatty acid synthetase complex show a higher activity towards β-ketoesters having a short chain alcohol moiety, such as methyl esters. An L-enzyme is more active on long chain counterparts. e.g.- octyl esters. As a consequence, the stereochemical direction of the reduction may be controlled by careful design of the substrate or by selective inhibition of one of the competing dehydrogenases.

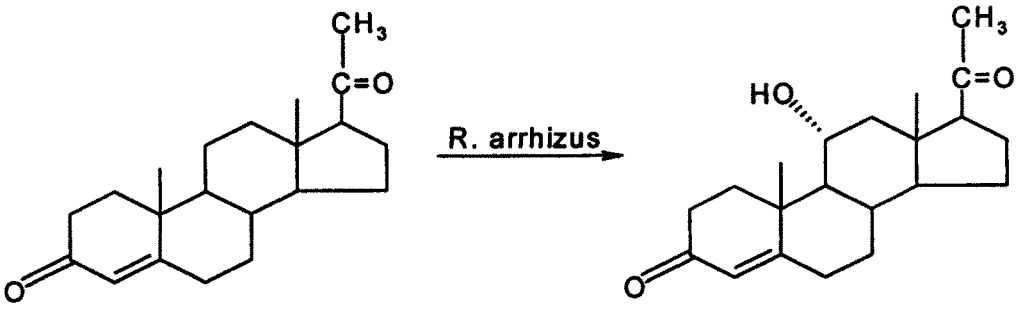
MICROBIAL TRANSFORMATION REACTIONS WITH RHIZOPUS ARRHIZUS

The utility of R-arrhizus in the biotransformation has been adopted by Peterson and Coworkers [52]. They have ~~been~~ obtained 11α-hydroxy progesterone from progesterone (5,1). The versatility of R arrhizus was not fully realized until the work of Holland et. al. [53,54] who carried out an exhaustive investigation with R. arrhizus ATCC 11145 with various steroidal substrates to bring about different types of chemical reactions such as 6β and 11α- hydroxylations, 4α-, 6α-, 6β-, 8α-, 8β- and 10β- allylic hydroxylations, 5, 6α-, 5, 6β-, 6, 7α-, 6, 7β epoxidatoins, inversions, dehalogenations, isomerisation of the double bonds, oxidations, reductions of the carbonyl functions (C=O) and hydrolysis of the acetate.

CHART - 5

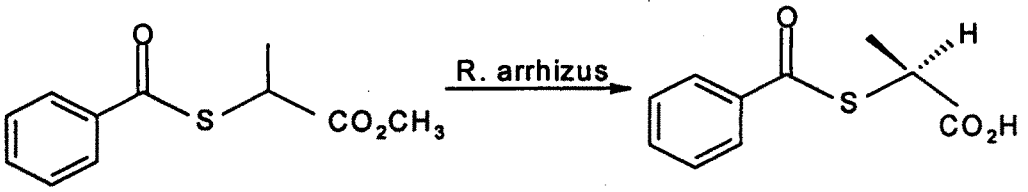
Scheme (5.1)

Microbial transformation of progesterone



Scheme (5.2)

Enentiospecific hydrolysis of (±) -methyl-3-benzoyl -3-thio-2-methyl propionate

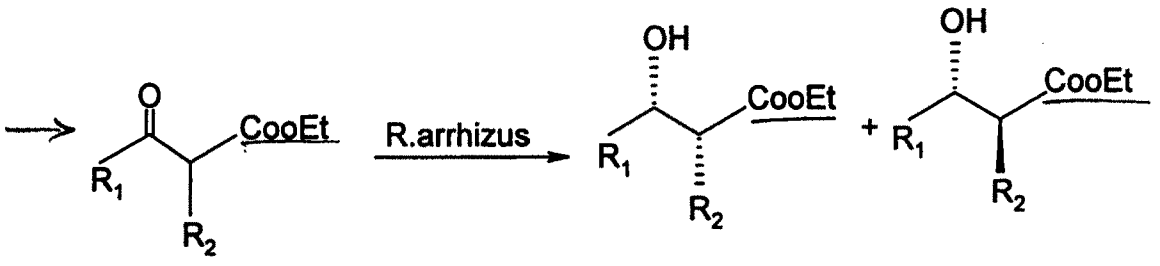


Yield 15%(S)-24%ee

CHART - 6

Scheme (6,1)

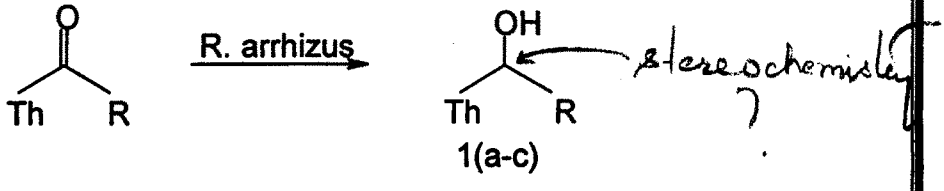
Microbial reductuion of α-alkyl-β-hydroxy esters



a=R_1=CH_3, R_2 = CH_3	Syn (2S, 3S)	anti
b=R_2=CH_3, R_2 = CH_3	64% (>95ee)	36% (>95ee)
	6%	94% (>99ee)

Scheme (6,2)

Microbial reduction of 2-acylthiazoles



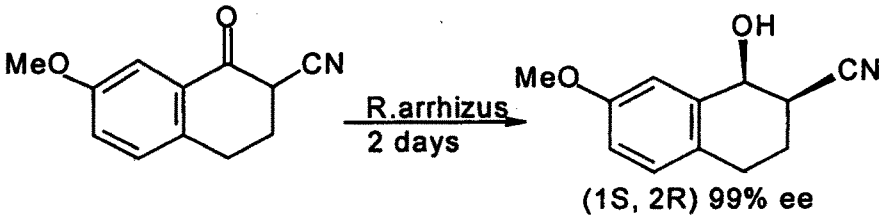
Th = 2-thiazolyl, a:R = CH_3, b: R=CH $(CH_3)_2$,

c: R = $CHCl_2$

CHART - 7

Scheme (7.1)

Microbial Reduction of 2-Cyano-1-Tetralones



(1S, 2R) 99% ee

Scheme (7.2)

R. arrhizus catalysed preparation of boththe enantiomers of sulcatol

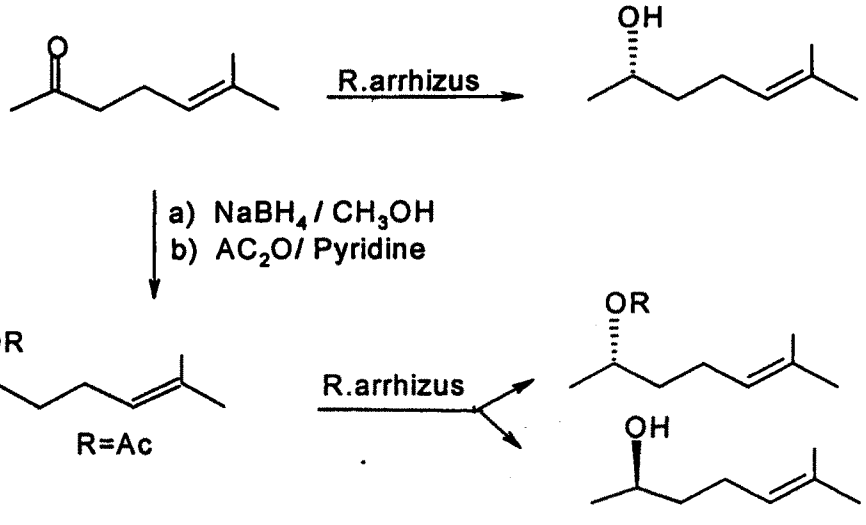
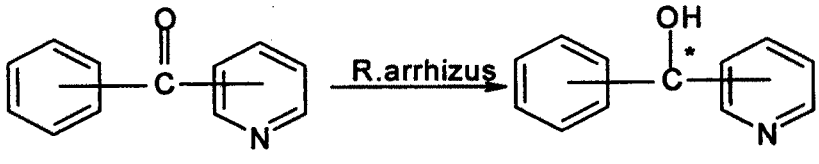
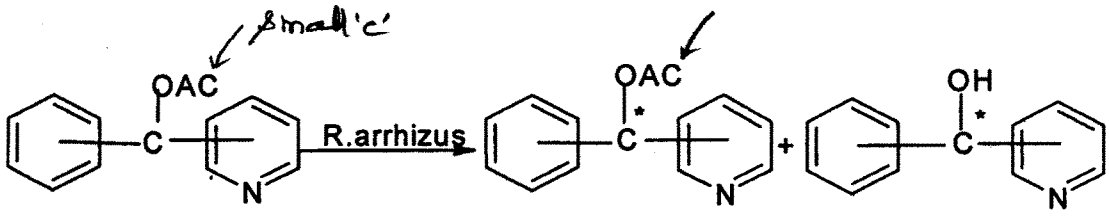


CHART - 8

Scheme (8,1)





Microbial transformations leading to (S) or (R) phenyl-pyridyl-methanol

Sih and Cowokers [55] reported simple method of preparing intermidate 3-benzoyl thio-2(5)-methyl propionic acid required in the preparation of an antihypertensive agent, captopril (5,2).

α-substituted β-hydroxy ester have wide applications in synthesis of biologically active substances. Azerad et al [56] reported R. arrhizus catalyzed reduction of 2-methyl 3-oxobutanoate or pentanoate to the corresponding anti – (2 S,3 S) – hydroxy esters in high optical purity (6,1).

The Fantin et al [57] have also exploited the same mould for the reduction of 20acylthiazoles. The resultant (R) - and (S) alcohols 1 (a-c) have been obtained in 30 - 95 % ee (6,2).

R. arrhizus is reported [58] to reduce methoxy substituted 2-cyano1-tetralones to cis-2-cyano-1-tetralones with high enantiomeric purity and yield (7,1).

Patil et al [59] reported short and simple method for the preparation of both the enantiomers of sulcatol an aggregation pheromone of the economical important pests of Gnathothrichus species (7,2).

Salvi et al [60] reported enantioselective synthesis of S-(+)-phenyl – pyridyl-methanols an analgesic and anticonvulsant using R.arrhizus in good optical purity (82% ee) (8,1).

Udupa et al recently reported sorption of radionuclides U^{233} and Pu^{239} at pH 6-7 by using biomass R. arrhizus as a sorbant for the treatment of radioactive effluents from nuclear industry.

The versatility of R. arrihizus is highlighted in this present review. The review survey reveals some lacunae in respect of its applications regarding reduction and hydrolysis reaction. It is of great interest to evaluate the potential of R.arrhizus so as to bring such a transformations on a wide array of substrate.

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