A Review on Biological Catalysts in Organic Synthesis

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Abstract: The application of biocatalysts for the synthesis of novel compounds has attracted increasing attention over the past few years and consequently, high demands have been placed on the identification of new biocatalysts for organic synthesis. The catalysis of many organic reactions reflects the importance and high expectations of this field of research. Enzymes play an increasingly important role as biocatalysts in the synthesis of key intermediates for the pharmaceutical and chemical industry, and new enzymatic technologies and processes have been established. Enzymes are an important part of the spectrum of catalysts available for synthetic chemistry. The synthetic applications of biocatalysts like oxidoreductases, transferases, hydrolases, lyases, isomerases and other natural biocatalysts obtained from fruits (coconut, pinapple and lemon) will be discussed in this review and exemplified by the syntheses of interesting compounds.

Keywords: Biological Catalyst, Organic Synthesis, Co-Factors, Enzymology, Fruits, Organic Compounds

I. INTRODUCTION

Organic synthesis is concerned with the construction of organic compounds from simple substances using known organic reactions. The use of enzymes (pure enzymes) or whole cells (those containing co-factors e.g. ATP, NAD, NADH, CoASH etc) as catalysts for chemical synthesis is known as biocatalysis [1]. Enzymes are proteins, and they are involved in virtually all transformations which take place *invivo*. They catalyze the transformations of many biologically importance molecules as well as reactions of substances which occur *in vitro* [1, 2, and 3]. As Chemistry turns more to the synthesis of complex substances which are derived from biologically important materials, a number of new methods such as enzymology, recombinant DNA technology, fermentation, tissue culture etc have become increasingly important part of the synthetic chemist's tools for producing chemical substances of interest [4, 5, and 6]. The general aim of the use of biocatalysts in organic synthesis is the formation of one stereoisomer of the chiral target compound. This type of synthesis is known as asymmetric synthesis.

One of the major challenges faced by synthetic chemists nowadays is the fact that different enantiomers of the same compound are usually produced during synthesis and these may have different interactions in biological systems. Consequently, the production of single enantiomers with specific activity, instead of racemic mixtures becomes an important issue in chemical industries e.g. pharmaceutical and agrochemical industries [7, 8]. This obstacle could be overcome by the use of biocatalysts (enzymes) since they are action specific.

Another set-back was fact that biocatalysts (enzymes) had no systematic method of nomenclature as many of their names did not convey enough information of the nature of the reactions they catalyzed and sometimes similar names were given to enzymes of different types. However, in 1956 the International Union of Biochemistry established the International Commission on enzymes which helped to solve the problem of enzymes nomenclature. The commission named enzymes based on

Enzyme Class (EC) classification system derived from the biochemical function of the enzyme in the living systems [9].

II. CLASSIFICATION OF ENZYMES (BIOCATALYSTS)

The table below shows the summary of the classification, reaction catalyzed, types and examples of enzymes.

Enzyme Class	*Reaction catalyzed*	*Enzyme type*	*Specific examples*
EC 1	Oxidation & reduction reactions	Oxidoreductases	Dehydrogenase, oxidase, oxygenase, perioxidase
EC 2	Transfer of a group from one molecule to another.	Transferases	Transaminase, glycosyltransferase, transaldolase.
EC 3	Hydrolysis reaction in water	Hydrolases	Lipase, protease, esterase, nitrilase, hydratase, glycosidase, phosphatase
EC 4	Non- hydrolytic bond cleavage	Lyases	Deoxycarboxylase, dehyratase, deoxyribosephosphate aldolase.
EC 5	Intermolecular rearrangement	Isomerases	Racemase and mutase
EC6	Bond formation requiring Triphosphate	Ligases	DNA ligase

Furthermore, the classification of enzymes is also based on the sub-classes which indicate the specific functional groups that are targeted during catalysis as shown below; [9, 10 and 11].

1. Oxido-reductases (oxidation-reduction reactions)

1.1 Acting on >CH─OH

1.2 Acting on >C=O

1.3 Acting on >C=CH─

1.4 Acting on >CH─NH_2

1.5 Acting on >CH─NH─

1.6 Acting on NADH & NADPH

2. Tranferases (transfer of functional groups)

2.1 One carbon groups

2.2 Aldehydic or ketonic groups

2.3 Acyl group

2.4 Glycosyl groups

2.5 Phosphate groups

2.6 Sulphur containing groups

3. Hydrolases (hydrolysis reactions)

3.1 Esters

3.2 Glycosidic bonds

3.3 Peptide bonds

3.4 Other C─N bonds

3.5 Acids anhydrides

4. Lysase (addition to double bond)

4.1 >C=C<

4.2 >C=O

4.3 >C=N─

5. Isomerase (isomerization reactions)

5.1 Racemases

6.Ligases(formation of bonds with ATP cleavage)

6.1 C─O 6.3 C─N

6.2 C─S 6.4 C─C

In biotransformation processes, about 60% of the biocatalysts used are the hydrolases, 20% are oxidoreductases while 20% is for the remaining four classes [11]. In the industry, the most commonly used biocatalysts are the proteases, lipases, esterases, amylases and amidases. With genetic

engineering, changes at the level of the enzyme can be made, altering its properties and leading to the formation of other varieties of the product. In addition, enzymatic engineering allows for the production of enzymes effective in a non-aqueous environment. This kind of environment is used in biocatalysis due to its interesting properties such as increased solubility of the substrate or hydrolytic reaction reversibility. Despite this, enzymes exhibit lower activity in a non-aqueous environment than in water. The addition of salt to the protein solution stabilizes its structure, which causes its greater activity. In this way, subtilisin can be activated as well as many other enzymes. In addition to salt, crown ethers, transition analogues and substrates, plus their copies, have an activating effect. This method is mainly used in the pharmaceutical industry [11, 12].

A) Advantages and Disadvantages of Biocatalysts

Similar to chemical catalysts, biocatalysts increase the speed of chemical reactions but do not affect the thermodynamics of the reactions. However, they offer some unique characteristics over conventional catalysts [12, 13].

The most important advantage of a biocatalyst is its high selectivity. This selectivity is often chiral (stereo-selectivity), positional (regio-selectivity), and functional group specific (chemo-selectivity). Such high selectivity is very desirable in chemical synthesis as it may offer several benefits such as non-usage of protecting groups, minimized side reactions, easier separation, and fewer environmental problems [14].

Other advantages include high catalytic efficiency and mild operational conditions. The characteristics of limited operating regions, substrate or product inhibition, and reactions in only aqueous solutions have often been considered as the most serious drawbacks of biocatalysts. However, many of these drawbacks turn out to be misconceptions and prejudices. For example, many commercially used enzymes show excellent stability with half-lives of months or even years under processed conditions. In addition, there is an enzyme-catalyzed reaction equivalent to almost every type of known organic reaction. Many enzymes can accept non-natural substrates and convert them into desired products. [15].

More importantly, almost all of the biocatalyst characteristics can be tailored with protein engineering and metabolic engineering methods to meet the desired process conditions. Biocatalytic processes are similar to conventional chemical processes in many ways. However, when considering a biocatalytic process one must account for enzyme reaction kinetics and enzyme stability for single-step reactions, or metabolic pathways for multiple-step reactions. Therefore, in a nutshell we can state that biocatalysis is a very important tool in organic synthesis because of the following reasons;

• Single steps in organic synthesis can be accomplished [14, 15, and 16].

• Preservation of stereochemical centers, which can be important for drugs

• Elimination of the need for protection or deprotection groups.

• Can be done in an aqueous environment – green chemistry

B) Enzyme Production

Although some enzymes are still extracted from animal or plant tissue, most of them are now produced from microorganisms by fermentation. Bacteria and fungi are the most popular hosts for producing industrial enzymes, due to easy handling and high productivity. They can also be readily genetically engineered to improve their performance; for example, by incorporating secretion systems to facilitate enzyme isolation and purification. Some of the most popular expression hosts are *Escherichia coli*, *Pichia pastoris*, *Pseudomonas fluorescens*, *Aspergillus sp*. and *Bacillus sp*. Mammalian or plant cells

are used in special cases. By regulation, the production host should have GRAS status (Generally Regarded as Safe Status) [17].

In a typical enzyme production procedure, cells containing genes encoding desired enzymes are grown in an Erlenmeyer flask. On a large-scale production, a computer-controlled fermenter or bioreactor is required to maintain an appropriate control of pH, O_2, NH_3 and CO_2 to maximize cell density. The cells are harvested by centrifugation in a batch or continuous fashion. Alternatively, they can be collected through membrane filtration devices. The cell membranes are then disrupted by an ultrasonicator or French press at small scale. At a scale of over 5–10 L, a homogenizer is usually used. After centrifugation to remove cell debris, the crude enzymes remain in the supernatant and can be concentrated through precipitation by adding either inorganic salts (e.g. ammonium sulfate) or organic solvents (e.g. acetone). The crude enzymes are then purified by dialysis or a variety of chromatographic methods. The dry powder is usually obtained after lyophilization under freeze-drying conditions [13, 17].

C) Immobilization of Enzymes

An enzyme is immobilized by attaching it to an insoluble support which allows its reuse and continuous usage, thus eliminating the tedious recovery process. Immobilization stabilizes the enzyme; moreover, two or more enzymes catalyzing a series of reactions may be placed in close proximity to one another. Adsorptions, covalent linkage, cross linking, matrix entrapment or encapsulation are different methods for making immobilized enzymes [17].

D) Scope of The Review

The availability of several publications in the literature clearly indicates the impact of biocatalysis in organic synthesis. Several excellent reviews are significantly available in this area. This write up is not intended to be, and it is not exhaustive as far as the application of biocatalyst in organic synthesis is concerned. However, it is only aimed at giving a general overview of the development reported in some of the articles based on the enzyme types, the reactions catalyzed and the specific examples of enzymes used on the various functional groups transformation as well as the products obtained. Also, included in this review are some of the works that are based on the application of fruit juice from coconut, pineapple and lemon and earthworm extracts as biocatalysts in organic synthesis.

III. BIOCATALYTIC REACTIONS AND APPLICATIONS

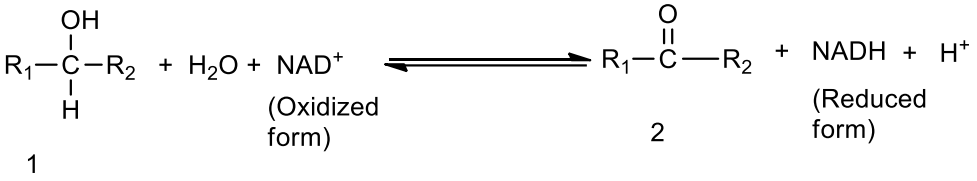
With respect to applications of enzymes in organic synthesis, enzymes in the all enzyme classes play an important synthetic role in organic chemistry, however, those from enzyme class 6 (ligases), have limited applications in organic syntheses. This is because *insitu* regeneration of the cofactor ATP is still a challenge, so that ligases have found limited use as catalysts for *in vitro* applications in organic syntheses. In contrast, enzymes from enzyme classes EC 1–5 turned out to be highly efficient catalysts for abroad range of organic synthetic transformations as well as suitable for technical-scale applications [11, 12].

A) OXIDOREDUCTASES

With oxidoreductases (EC 1) many successful reduction and oxidation processes have been realized. However, with respect to (asymmetric) reductions as a synthetically important reaction in organic chemistry, the reduction of a carbonyl moiety to an alcohol (when using, for example, alcohol dehydrogenases or α- hydroxy acid dehydrogenases as catalysts) or amino functionality (when using α- amino acid dehydrogenases in reductive aminations) has already found a wide range of applications in organic chemistry as well as in industrial operations. In addition to hydroxylation, other oxidative processes with enzymes are also of interest in organic syntheses, such include reactions with Baeyer–Villiger monooxygenases (for Baeyer–Villiger oxidations leading to lactones from ketones) and

styrene monooxygenases (for epoxidation of styrenes) [17, 18]. Oxidoreductases are the second most used enzyme types in organic synthesis

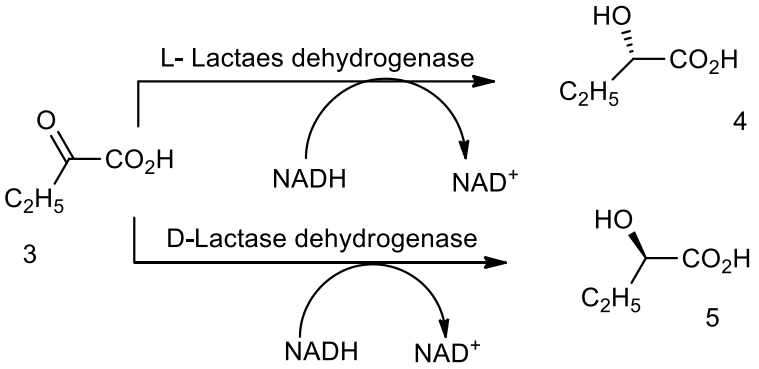
Branden *et al*, [18] reported that carbonyl compounds (2) can be produced from alcohols (1) when alcohol dehydrogenase, from *Candida parapsilosis* containing the oxidized form of nicotinamide adenine dinucleotide (NAD^+) is used as a catalyst. This reaction is reversible, as the carbonyl dehydrogenase containing the reduced form of nicotinamide adenine dinucleotide NADH can convert carbonyl compounds to their corresponding alcohols as outlined in the scheme 1 below;



R_1, R_2 = Alkyl or aryl groups

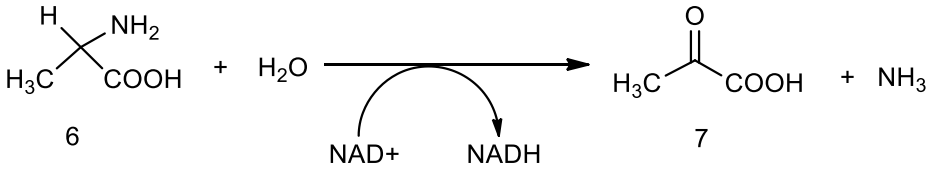
Scheme 1

In line with the above Alan *et al* [19] described the transformation of 2-oxobutanioc acid (3) to stereospecific isomers of α-hydroxybutanioc (4) and (5) acid using the L and D-Lactase dehydrogenase respectively as shown in scheme 2 below;



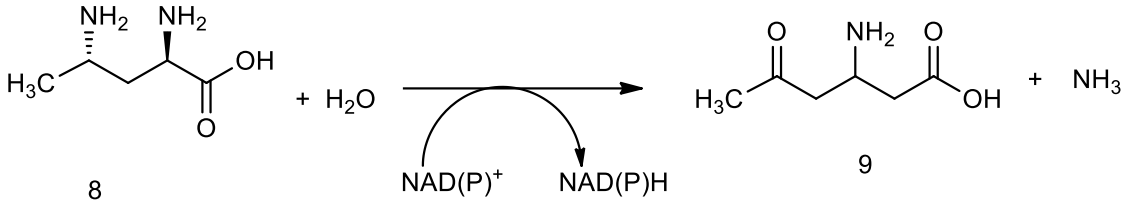
Scheme 2

The same authors [19] also described the catalytic action of α- amino acid dehydrogenase in reductive amination of amino functionality as observed in the reduction reaction of L-alanine (6) by L-alanine dehydrogenase EC 1.4.1.1 from *Bacillus cereus* to an α-carboxylic acid (7) as shown in scheme 3 below;



Scheme 3

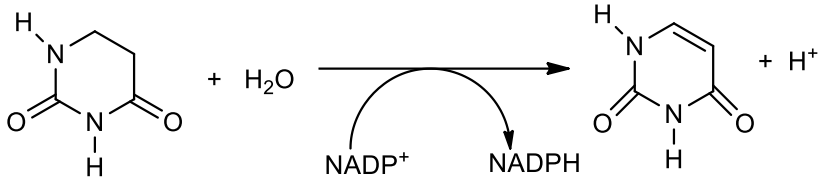
Similarly, 2, 4-diaminopentanoate dehydrogenase was reported to reduce 2, 4-diaminopentanioc acid (8) to 2, 4-amino -4-oxopentanioc acid (9) by the same authors [19] as shown in scheme 4 below;



8 9

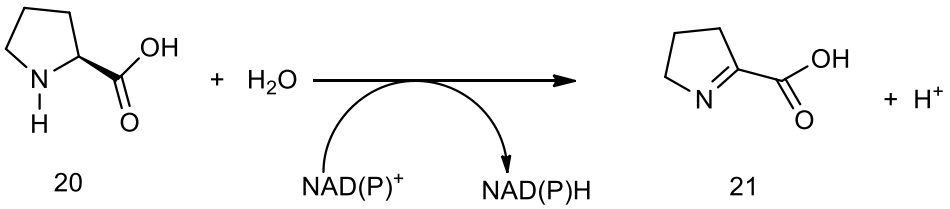
Scheme 4

It was observed by Alan *et al* [19] that oxidoreductases can also reduce substrates containing -CH=CH- group as well as those containing -CH-NH groups as seen in the reduction of 5, 6-dihydrouracil (18) to uracil (19) by dehydropyrimidine dehydrogenase and the reduction of proline (20) by pyroline-5-carboxylate reductase to 1-pyrollin-2-carboxylic acid (21) as shown in the schemes 8 and 9 below;



18 19

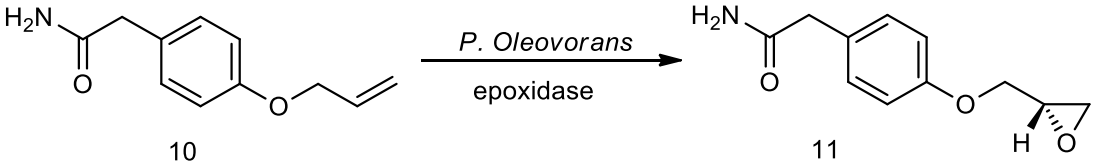
Scheme 8



20 21

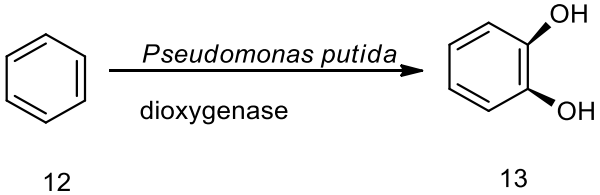
Scheme 9

Other examples of reaction catalyzed by oxireductases include epoxidation of alkene, hydroxylation of benzene using epoxidase and dioxygenase as well as the lactonization of cyclohexanone by monooxygenase respectively according to Grace Desantis [20] as shown in the schemes 5, 6 and 7 below;



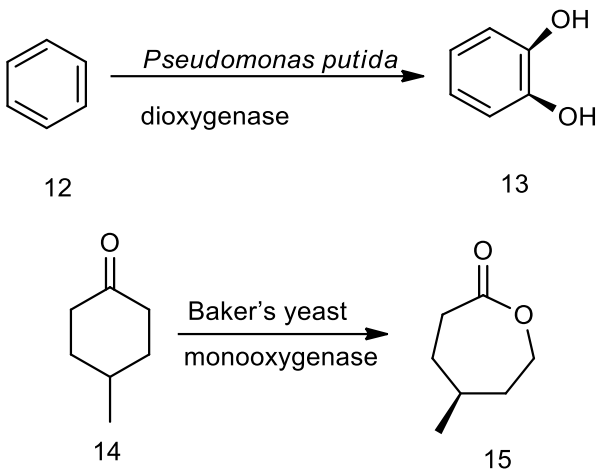
10 11

Scheme 5



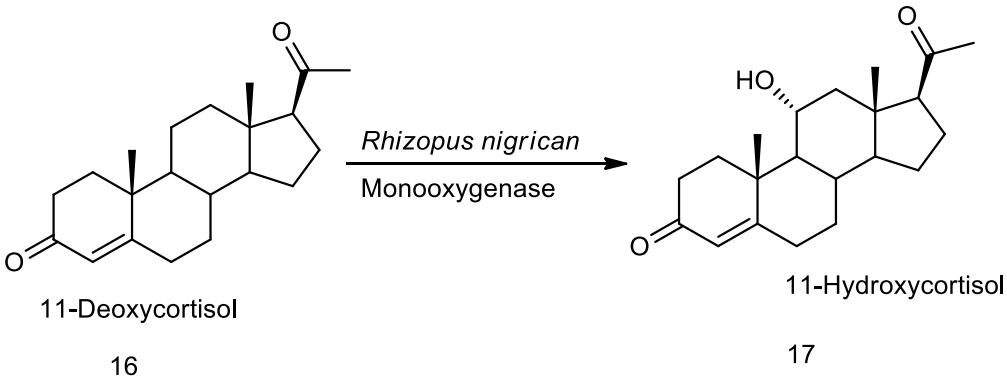
12 13

Scheme 6

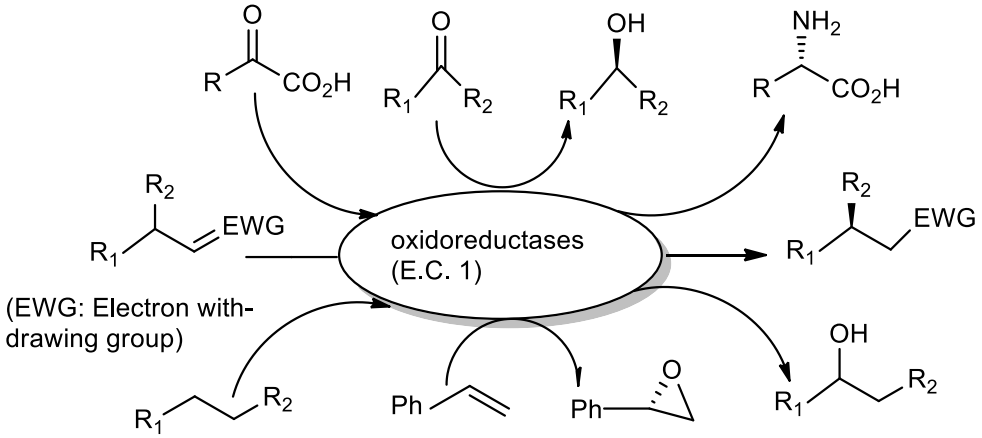


Scheme 7

Peterson and Murray at the Upjohn Company discovered a commercially viable synthetic route of synthesizing cortisol that replaced a 31-step chemical synthesis from a bile acid and this paved the way for the subsequent commercial success of the steroid hormones [20, 21] The carticosteriod, cortisol (17) is useful medicine for the treatment of arthritis and it can be made from the cheap precursor 11-deoxycortisol (16) using 11β-monooxygenase as shown in scheme 8 below.



On a general note, scheme 9 below shows the overview of selected reactions catalyzed by enzymes from EC 1 (oxidoreductases) that have gained broad interest in organic synthesis [11]

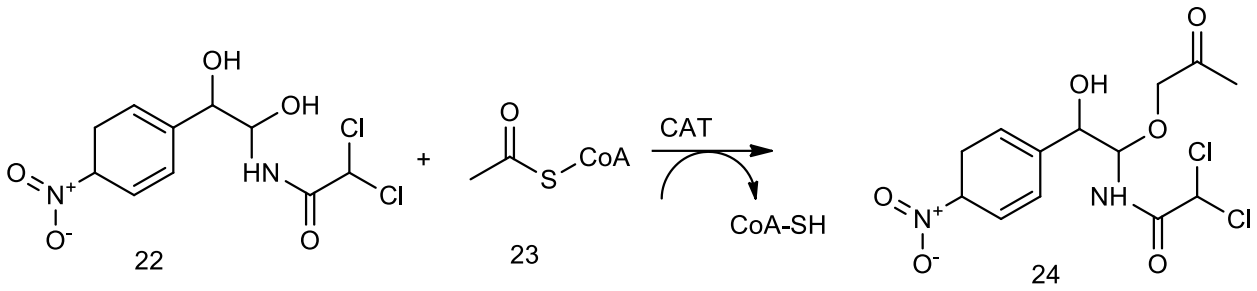


Scheme 9

B) TRANSFERASES

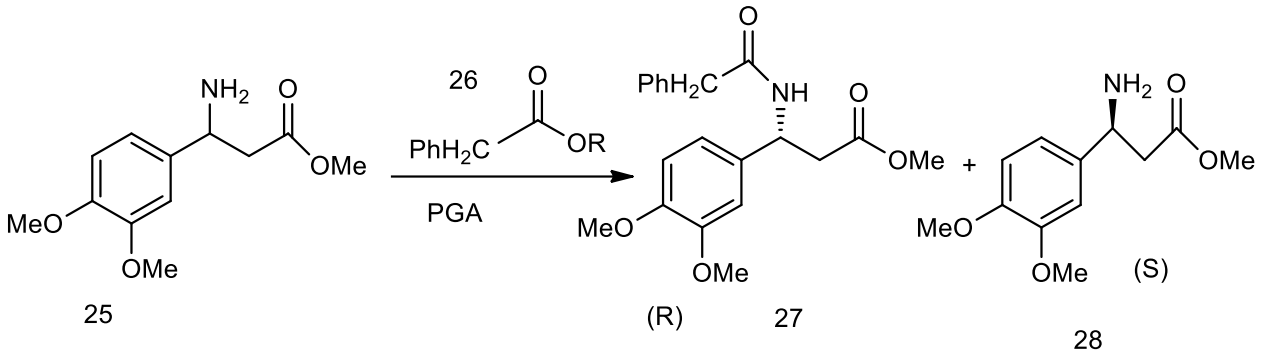
Representatives of enzyme class EC 2, the transferases, are also versatile catalysts for organic synthetic transformations. In particular, transaminases have attracted widespread attention with interesting applications for the synthesis of amino acids and amines. Industrial applications have been reported as well. Transferases catalyze the transfer of groups such as acyl, sugar, phosphoryl, and aldehyde or ketone moieties from one molecule to another. [20, 21]

Annika et *al* [22] demonstrated that the acylation of chloramphenicol can be catalyzed by *chloramphenicol acetyltransferases* (CAT) via the transfer of the acetyl group from *acetyl-CoA* (**23**) to the primary hydroxyl group of chloramphenicol (**22**) to form 3-acetylchloramphenicol (**24**) as shown in 10 scheme;



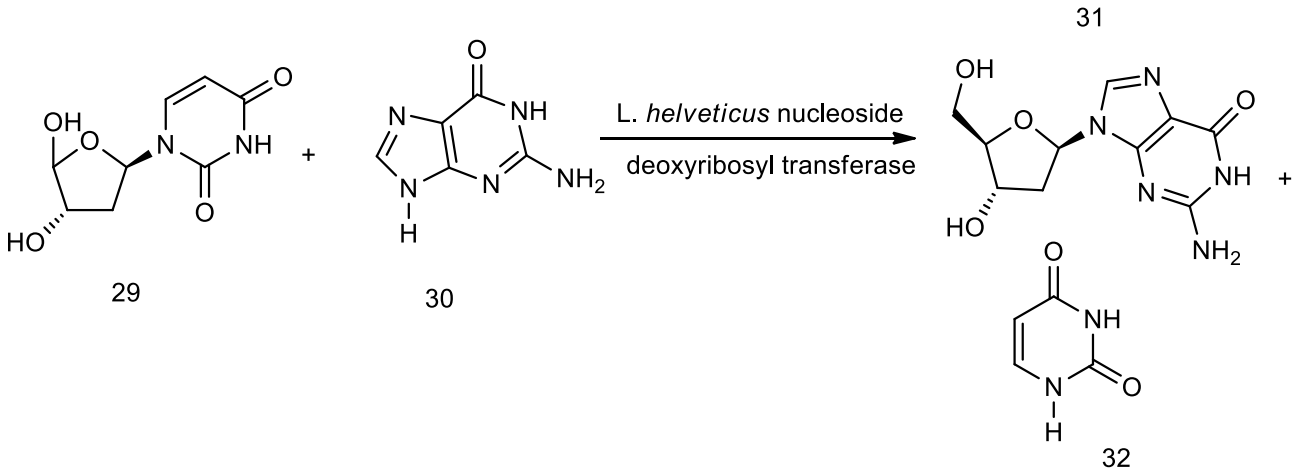
Scheme 10

Benjamin *et al* [23] showed that acyl transferases can perform enantioselective transfer reactions and also catalyze the formation of a wide range of esters and amide bonds as shown in scheme 11 below;



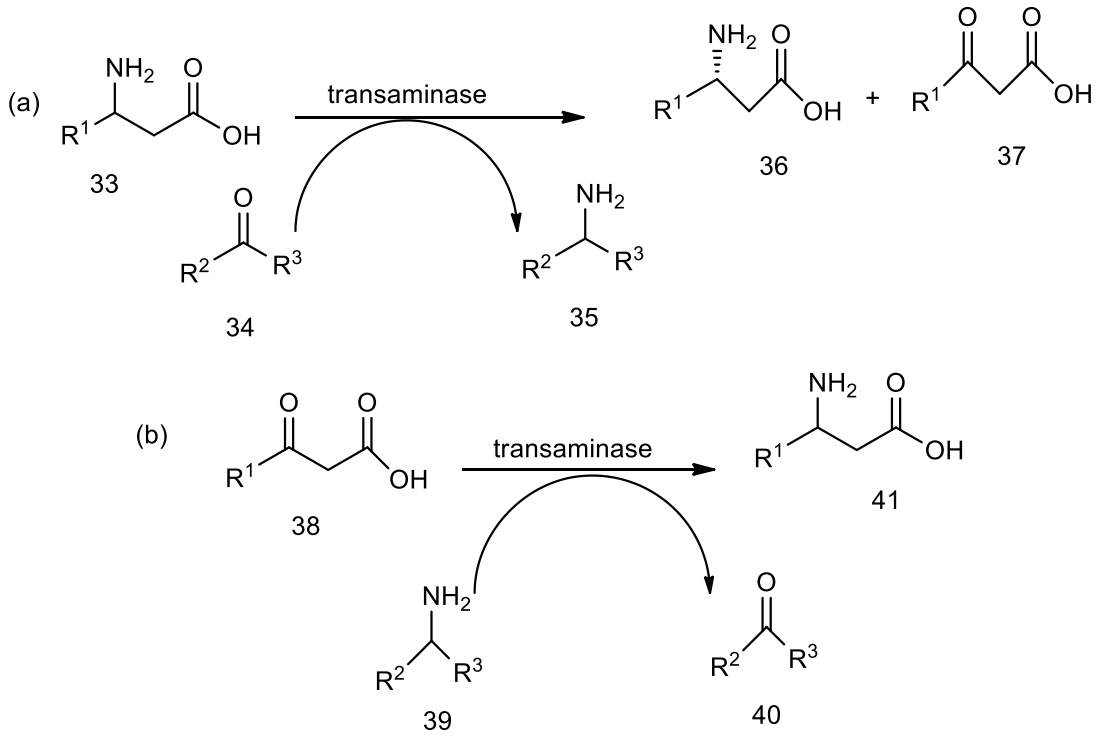
Scheme 11

According to the authors [20-24], the preparation of nucleosides analogues (antiviral precursors) can be catalyzed by glycosyl transferase (deoxyribosyl tranferase). This reaction involves the transfer of a sugar group from compound (**29**) to (**30**) to formed a nucleoside (**31**) as shown in scheme 12 below;



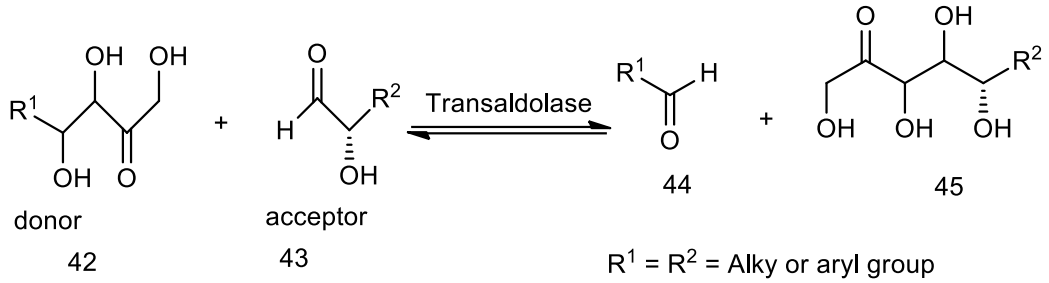
Scheme 12

The transfer of an amino group is catalyzed by transaminase. This process is used for the preparation and resolution of amino acids and their analogues. As a starting material, the corresponding carbonyl compounds are required. Jen *et al* [24] noted that TAs can be applied either in the kinetic resolution of racemic β-amino acids or in asymmetric synthesis of amino acids, starting from the corresponding prochiral β-keto-substrate. Scheme 13 (a) and (b) respectively, below illustrates the above processes.



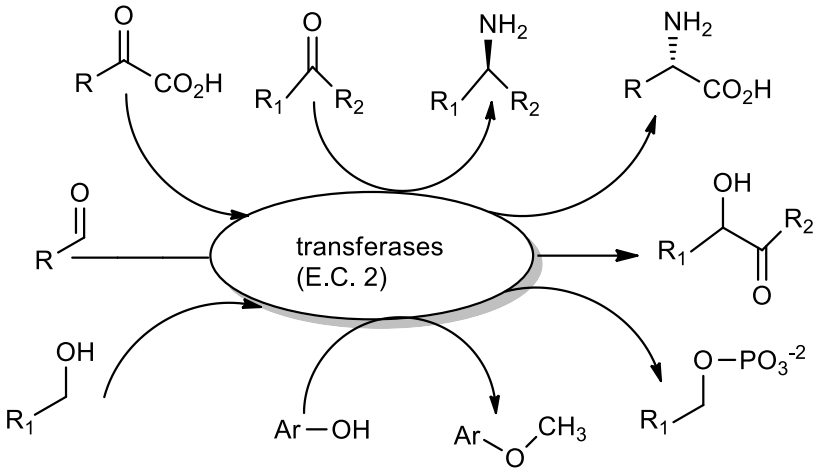
Scheme 13 (a) and (b)

Still in this series of transferases, scheme 14 shows the transfer of a dihydroxyacetone moiety (ketone) derived from a donor substrate to an acceptor substrate catalyzed by Transaldolase EC 2.2.1.2 from *E. coli* [24, 25].



Scheme 14

Scheme below 15 shows the overview of selected reactions catalyzed by enzymes from EC 2 (Transferases). [11]

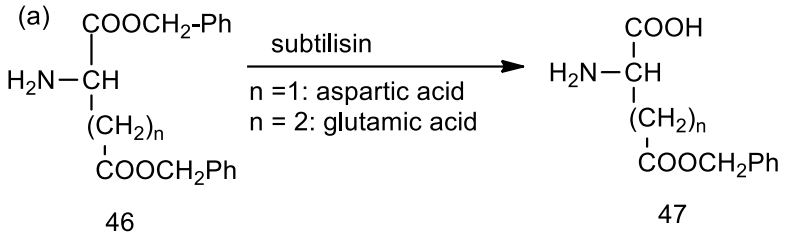


Scheme 15

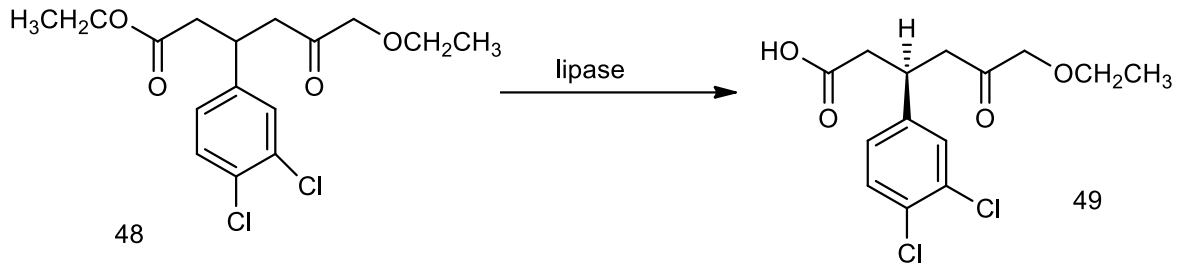
C) ***HYDROLASES***

Hydrolases (EC: 3) catalyze the hydrolytic cleavage of glycosides, anhydrides, esters, amides, peptides, and other C–N moieties. These reactions are referred to as hydrolysis. Below are some of the transformations that are carried out by this group of biocatalysts.

Tyler *et al* [16] reported that proteases such as α-chymotrypsin, papain, and subtilisin are useful biocatalysts for region-selective or stereoselective hydrolytic biotransformations. For example, dibenzyl esters of aspartic and glutamic (**46**) acid and other related compounds can be selectively deprotected at the 1-position to give their derivatives (**47**) by subtilisin-catalyzed hydrolysis as shown in schemes 16 a and 16 b below respectively;

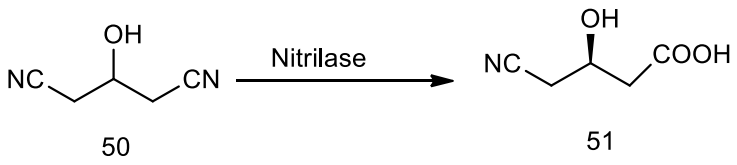


(b)



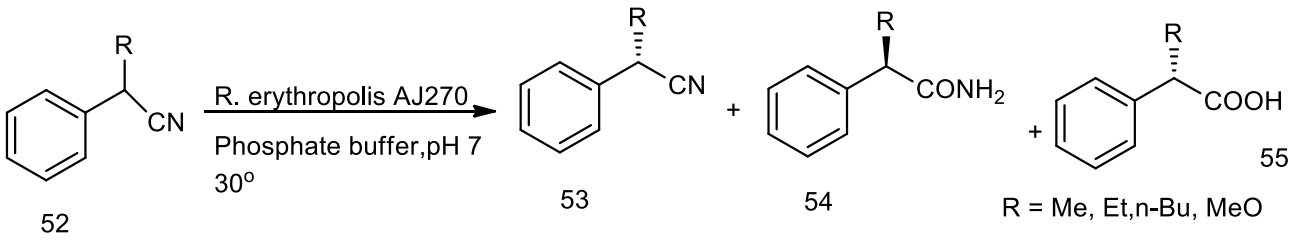
Scheme 16(a) and 16(b)

In addition to the proteases and the lipases, the nitrilases also play an important function in the preparation, resolution and the conversion of the nitrile groups to acid groups as shown in schemes 17, 18 and 19 below;

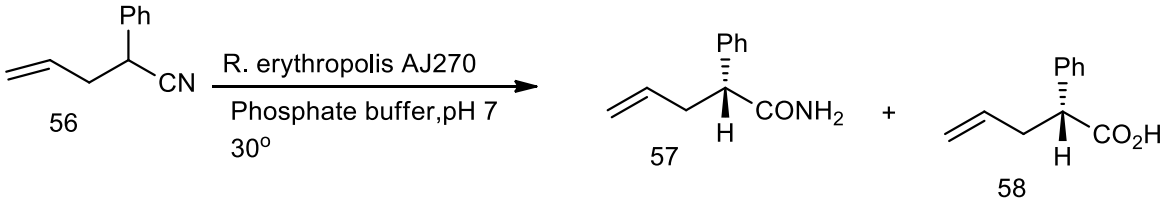


Scheme 17

The same authors above demonstrated that *Rhodococcus sp* AJ270 containing a nitrilase was able catalyzed the stereoselsctive conversion of α- substituted phenylacetonitriles under mild conditions into amides and carboxylic acids as shown in the scheme 18 below;

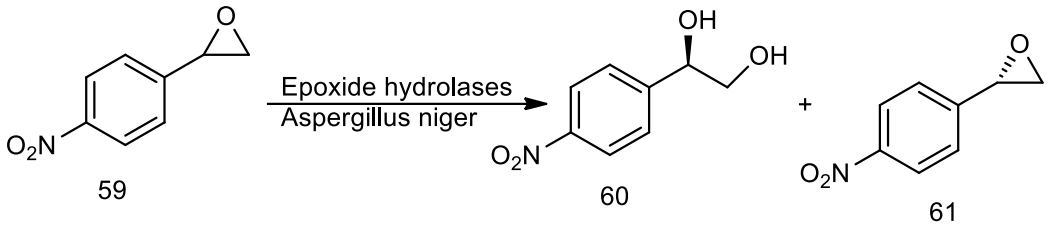


Scheme 18



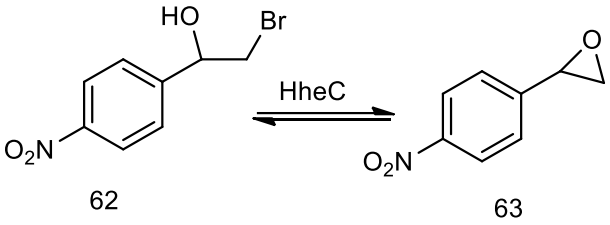
Scheme 19

Furthermore, Grace Desanti [20] stated that hydrolases for example the epoxide hydrolases can catalyze the resolutions of epoxides as well as their conversion to the glycols as shown in scheme 20.



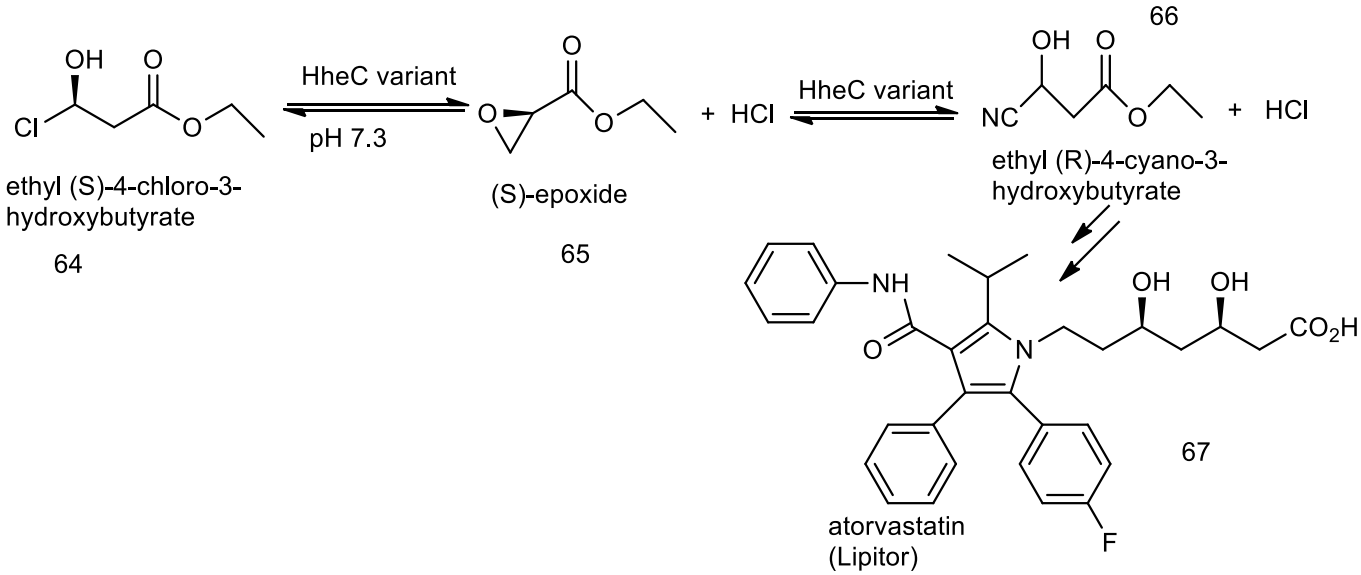
Scheme 20

In another development report was also given by Geoffrey A. Behrens *et al* [11] on the use of halohydrin dehalogenases HheC to catalyzed the synthesis of epoxides (**63**) from halogenated substrates (**62**) as shown in the scheme 21 below;



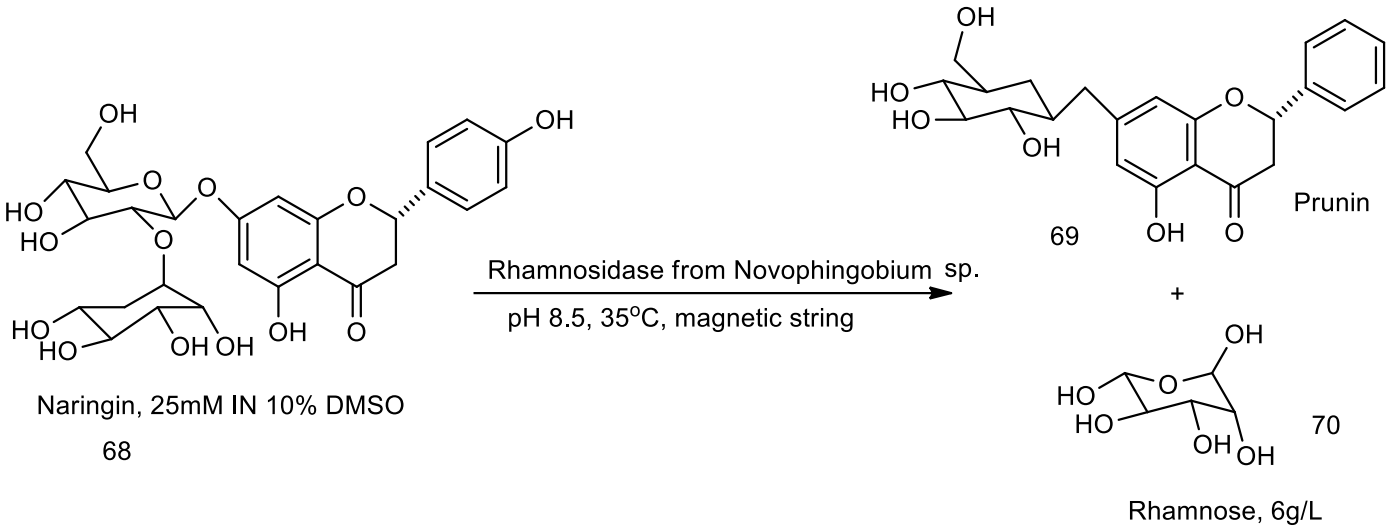
Scheme 21

The author also described (scheme 22) below how a halohydrin dehalogenase HheC variant from *Agrobacterium radiobacter* was used to catalyzed the highly selective formation of ethyl (*R*)-4-cyano-3-hydroxybutyrate (66) from the (*S*)-chloro derivative, (**64**) which can be subsequently used in the preparation of atorvastatin (**67**).



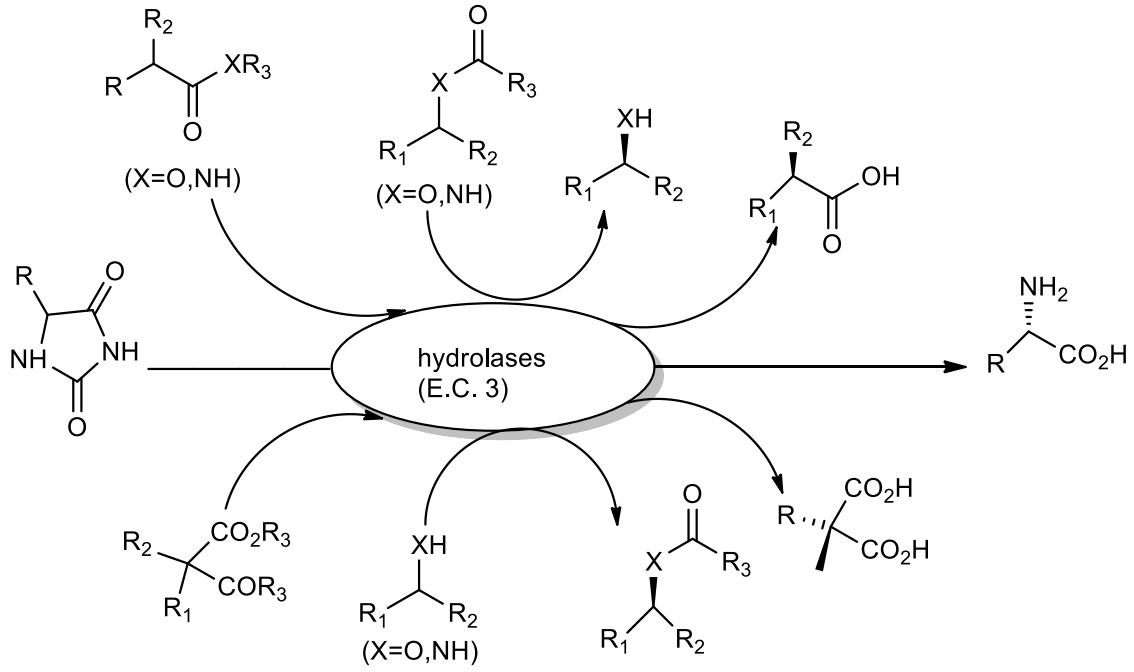
Scheme 22

Furthermore, the above authors described the hydrolytic transformation of narigin, to prunin and rhamnose (scheme 23) under the influence of a glycosidase known as rhamnosidase from *Novosphingobium spp* in the reaction below. The experimental conditions involve optimum alkaline pH of the enzyme and 125 mM naringin solution, to produce prunin with a yield of 32.1% as well as free L-rhamnose as a secondary product at a concentration of 6 g/L.



Scheme 23

Scheme 24 below shows the overview of selected reactions catalyzed by enzymes from EC 3 (Hydrolases).



Scheme 24

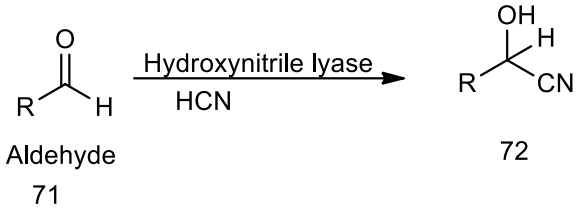
D) LYASES

Lyases (EC: 4) catalyze additions, usually of HX, to double bonds such as C=C, C=N, and C=O as well as the reverse processes.

Monica *et al* [25] stated that hydroxylnitrile lyases are used to catalyze the synthesis of chiral hydroxy nitriles (cyanohydrins) which can be used to make chiral hydroxyl acids (scheme 24).

The same authors [25] observed that hydrogen cyanide is the most preferred cyanide source in cyanohydrins synthesis (scheme 25). Besides HCN, several different cyanide sources like potassium

cyanide can be used as well in the biotransformation. Alternatively, the addition of hydrogen cyanide in the reaction can be replaced by its indirect generation by addition of the acid to the aqueous solution of alkali cyanide in trans- hydrocyanation process. This slow diffusion of HCN gives advantage over spontaneous addition and results in high enantiomeric purity and yield.

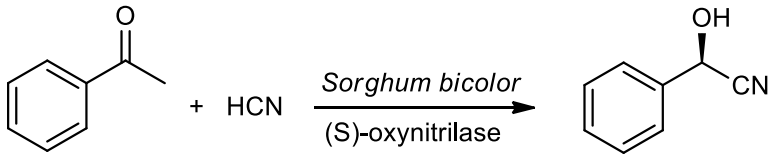


Aldehyde
71

72

Scheme 25

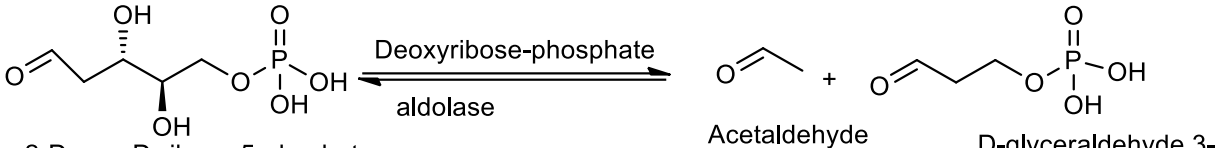
In line with the above observation Grace Desanti [20] reported the biotransformation of phenylethanone to 2-hydroxyl-2-phenylnitrile (scheme 26) through the catalytic activity of s-oxynitrilase from *Sorghum bicolor.*



73 74

Scheme 26

Rachel *et al* [26] described the catalytic ability of a lyase *deoxyribose-phosphate aldolase* on 2-deoxy-D-ribose 5-phosphate (75) to give acetaldehyde (76) and D-glyceraldehyde 3-phosphate (**77**) as shown scheme 27 below;



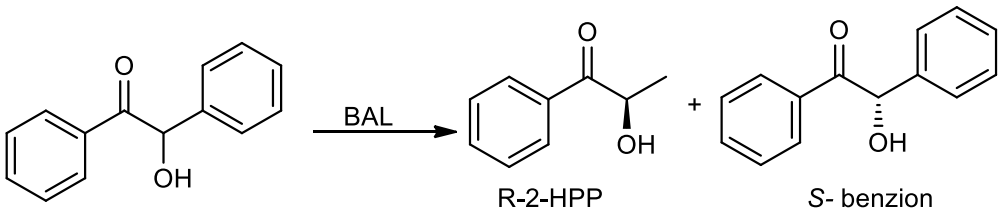
2-Deoxy-D-riboes 5-phoshate
75

Acetaldehyde
76

D-glyceraldehyde 3-phosphate
77

Scheme 27

The above authors (26) also reported on the use of benzaldehyde lyase (BAL) to catalyze the transformation of *rac*-benzion to *R*-2-hydroxylphenylpropanone as well as its resolution to *S*- benzion in scheme 28 below;



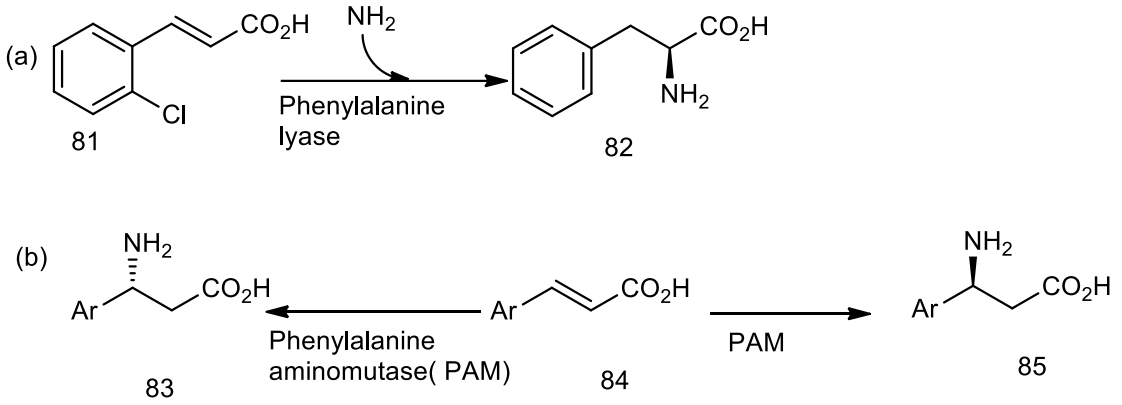
rac- benzion
78

R-2-HPP
ee > 99%
79

S- benzion
ee > 99%
80

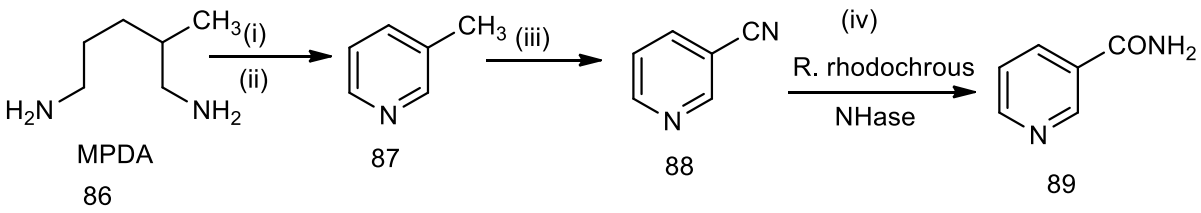
Scheme 28

Furthermore, the same authors above also reported on the use of ammonia lyases as efficient biocatalysts for biotransformation, by describing the action of phenylalanine lyase and phenylalanine aminomutase in the synthesis of amino acids as shown in schemes 29a and 29b below respectively;



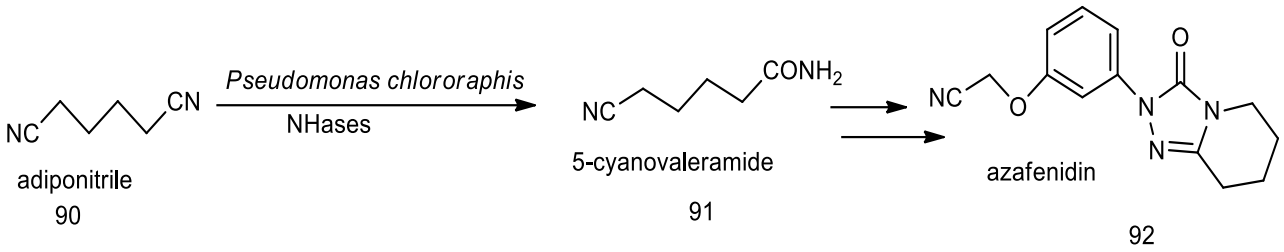
Schemes 29a and 29b

Sander Van Pelt [27] reported that a lyase known as nitrile hydratase (NHase) was used in the production process of nicotinamide (niacinamide, vitamin B3) (scheme 30). The process involves four highly selective, continuous catalytic reaction steps namely (i) cyclization, (ii) dehydrogenation (iii) ammoxidation and (iv) enzymatic hydration using NHase. The starting material is 2-methylpentanediamine, (**86**) which is a by-product obtained from nylon-6, 6 production. The last step which is the hydration of 3-cyanopyridine (**88**) to nicotinamide, (**89**) is carried out by using *R.rhodochrous* J1 whole cells (containing NHase) immobilised in polyacrylamide gel particles.



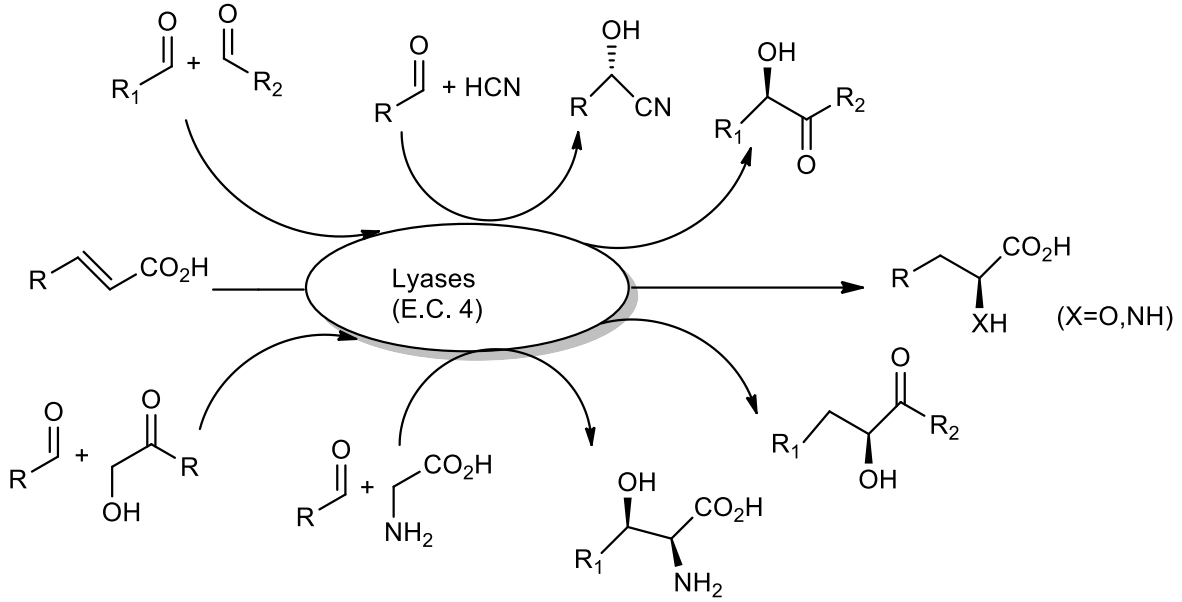
Scheme 30

The author [27] above also reported that the production of 5-cyanovaleramide (5-CVAM) (**91**) which is an intermediate for the production of the herbicide, azafenidin (**92**) from adiponitrile (**90**) was achieved by using the regioselective properties of NHase, 5-CVAM. It was produced by DuPont using immobilised *Pseudomonas chlororaphis* B23 cells containing NHase in high conversion (97%), high yield (93%) and high selectivity (96 %). They concluded that the use of a biocatalyst in the above reaction resulted in higher yields, higher catalyst productivity, less by-product formation, and generates significantly less process waste than the alternative chemical methods which make use manganese dioxide as a catalyst.



Scheme 30

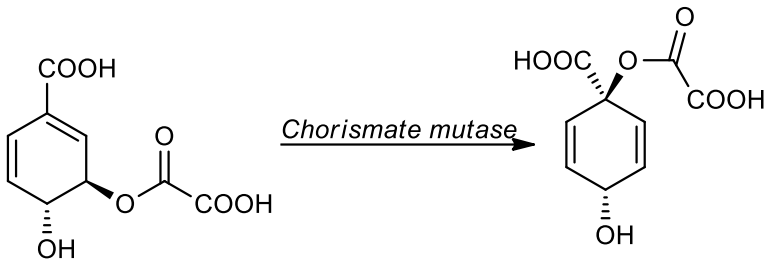
Scheme 31 below shows the overview of selected reactions catalyzed by enzymes from EC 3 (Lyases) [11]



*E) **ISOMERASE***

Enzyme class EC 5 consists of those enzymes capable of catalyzing isomerization reactions. The types of isomerizations are diverse, consisting of, for example, racemizations, 1, 2-migrations of functional groups (e.g. of amino functionalities) and cis–trans isomerizations. In organic chemistry, the use of racemases has attracted most interest within the enzymes of EC 5, since the combination of a racemase with another biocatalyst for a resolution step enables the development of dynamic kinetic resolution processes. Typically, such resolution processes to be combined with racemases are reactions catalyzed by hydrolases, and such resolutions are run either in the hydrolytic or acylation direction [26, 27].

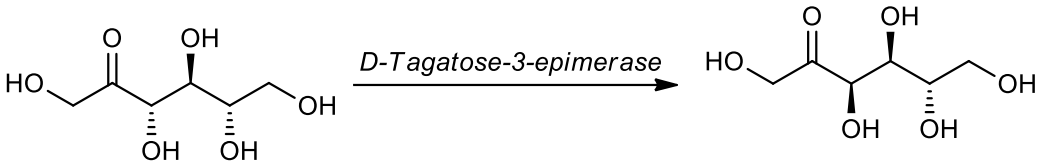
Below are some of the reactions catalyzed by the isomerases given by the above authors.



93 94

Scheme 32

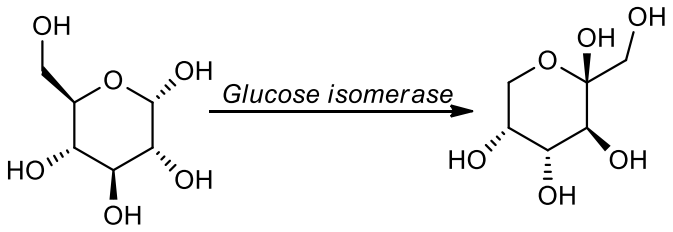
The sub-class epimerases catalyze the empimerization of compounds [27] (i.e. the changing of one epimeric compound to another by enzymatic actions) .This is used for the preparation of epimers as shown in scheme 33 below;



95 96

Scheme 33

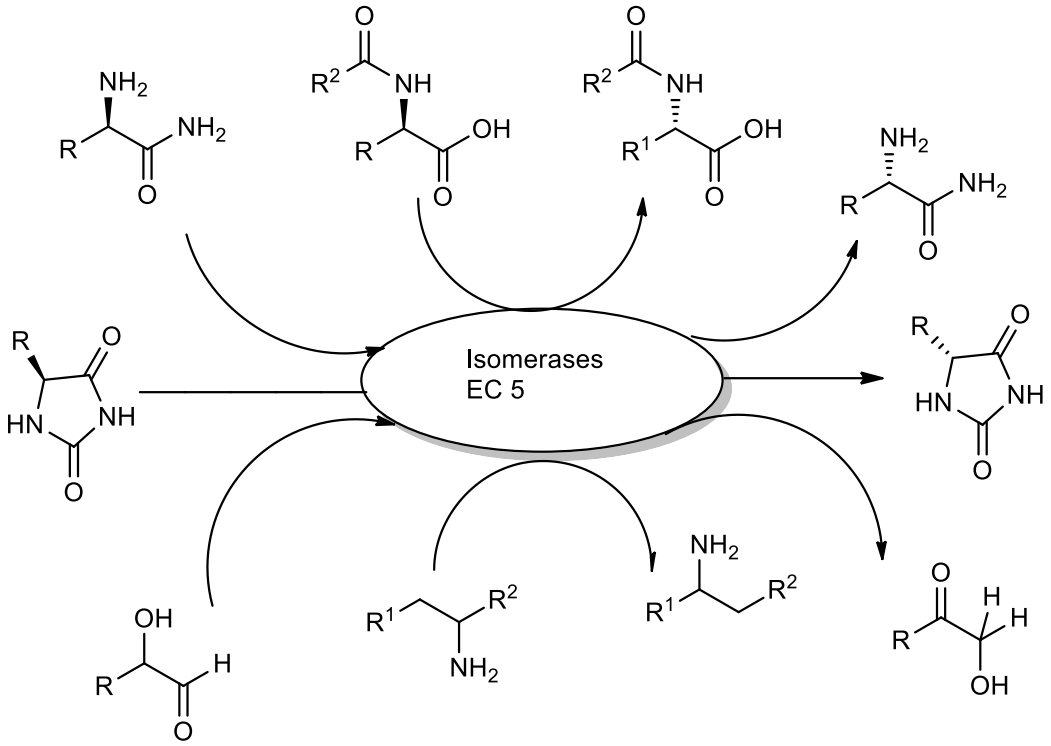
Interestingly, the largest biocatalytic application of isomerase today is based on the use of an isomerase, namely *glucose isomerase*, for the production of high fructose corn syrup via enzymatic transformation of glucose into fructose [27-28] as shown in scheme 34 below;



97 98

Scheme 34

Scheme 35 below shows the overview of selected reactions catalyzed by enzymes from EC 5 (isomerases) that have gained broad interest in organic synthesis [11]



Scheme 35

F) LIGASES

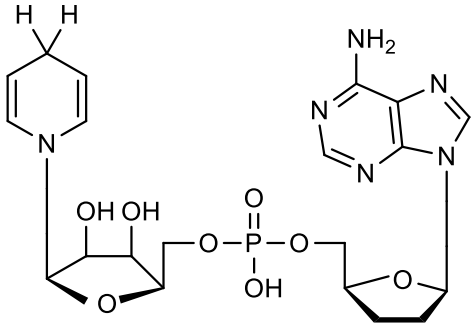
Ligases (EC: 6) catalyze the formation of C–O, C–S, C–N, C–C, and phosphate ester bonds. [29, 30 31] These enzymes are also known as synthetases. Whereas enzymes from enzyme classes EC 1 to EC 5 are already widely used as catalysts in organic synthesis and have enabled a broad range of highly efficient synthetic processes, the application range of enzymes from EC 6 (ligases) is still narrow. At first glance this might sound surprising due to the numerous interesting reaction types these enzymes can catalyze. However, these reactions require ATP as a cofactor, which is efficiently regenerated in living cell processes, but its cofactor regeneration *in situ* under *in vitro* reaction conditions remains a challenge. Although some methods have been developed, applicability in organic syntheses (in particular with respect to large-scale processes) is still limited. [30]

G) COFACTORS AND CO-ENZYMES

Cofactors are non-proteinogenic compounds that are required for the catalytic activity of enzymes. They can bind to the enzyme either in a covalent or non-covalent mode. Abroad variety of cofactors is known, consisting of organic molecules and inorganic ions. A cofactor that is covalently bound permanently to the enzyme is called a prosthetic group while that which is non-covalently bound to the enzyme it is called a coenzyme. The modification of a coenzyme during the catalytic process is determine either by the transferring of electrons or chemical groups to the substrate, therefore, its regeneration in a subsequent reaction is a major factor to consider in its usage in catalytic amounts. Consequently, the co-substrate required for the cofactor's regeneration must also be in stoichiometric amount [30, 31].

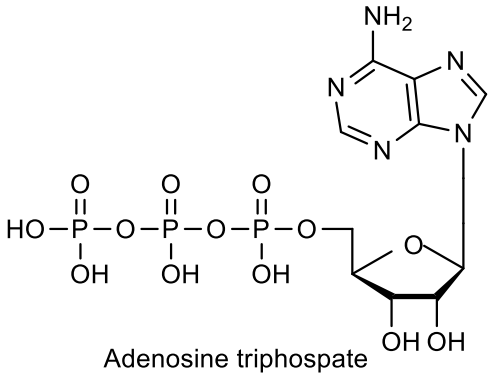
Members of all other enzyme classes in most cases show a cofactor dependency; with exception of the hydrolases (EC 3) although in some cases in the lyases (EC 5) cofactors are not necessarily involved in the catalytic process. The determining factor of the choice of a co-factor and its attractiveness in a synthetic process depends much on the ease to regenerate such cofactors efficiently under given organic reaction conditions. In order to deal with the issue of cost effectiveness, *insitu*

cofactor regeneration is also a prerequisite to consider when carryout biocatalytic processes in a synthetically useful and attractive fashion. This is because most cofactors for example NAD(P)H and its oxidized form, $NAD(P)^+$ [30, 31] which are used in enzymatic redox processes are very expensive. Such in situ cofactor regeneration can be achieved through combination with a second enzymatic transformation, which regenerates the cofactor. To make the cofactor regeneration economically attractive it is important that the substrate consumed in this second enzymatic process is cheap and readily available. The scheme below shows selected cofactors that are often applied in organic synthetic processes with enzymes.



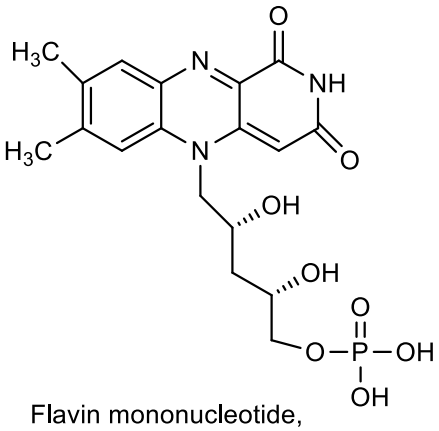
Nicotinamide adenonsine dinucleotide phosphate NAD(P)H

99



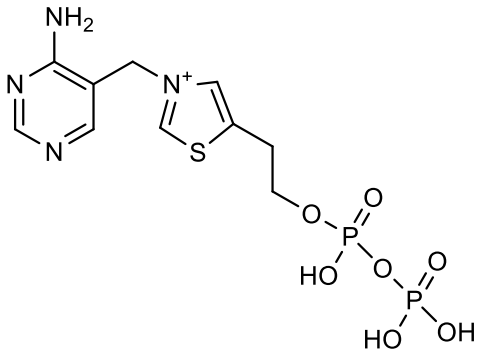
Adenosine triphospate

100



Flavin mononucleotide, FMN

101

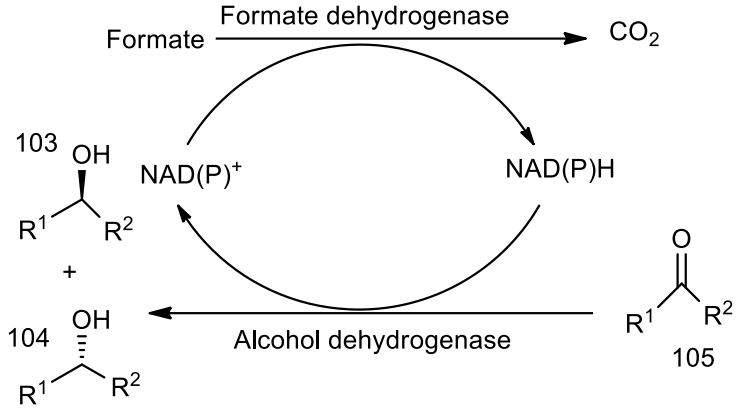


Thiamine diphosphate, ThDP

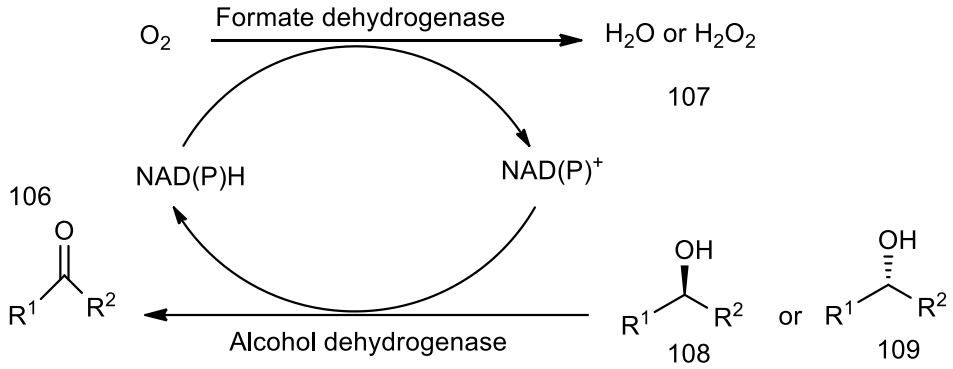
102

The schemes below are examples of the activities of the reductive and oxidative cofactors involved in biotransformation processes.

(a) Reductive cofactor recycling pattern of formate dehydrogenase



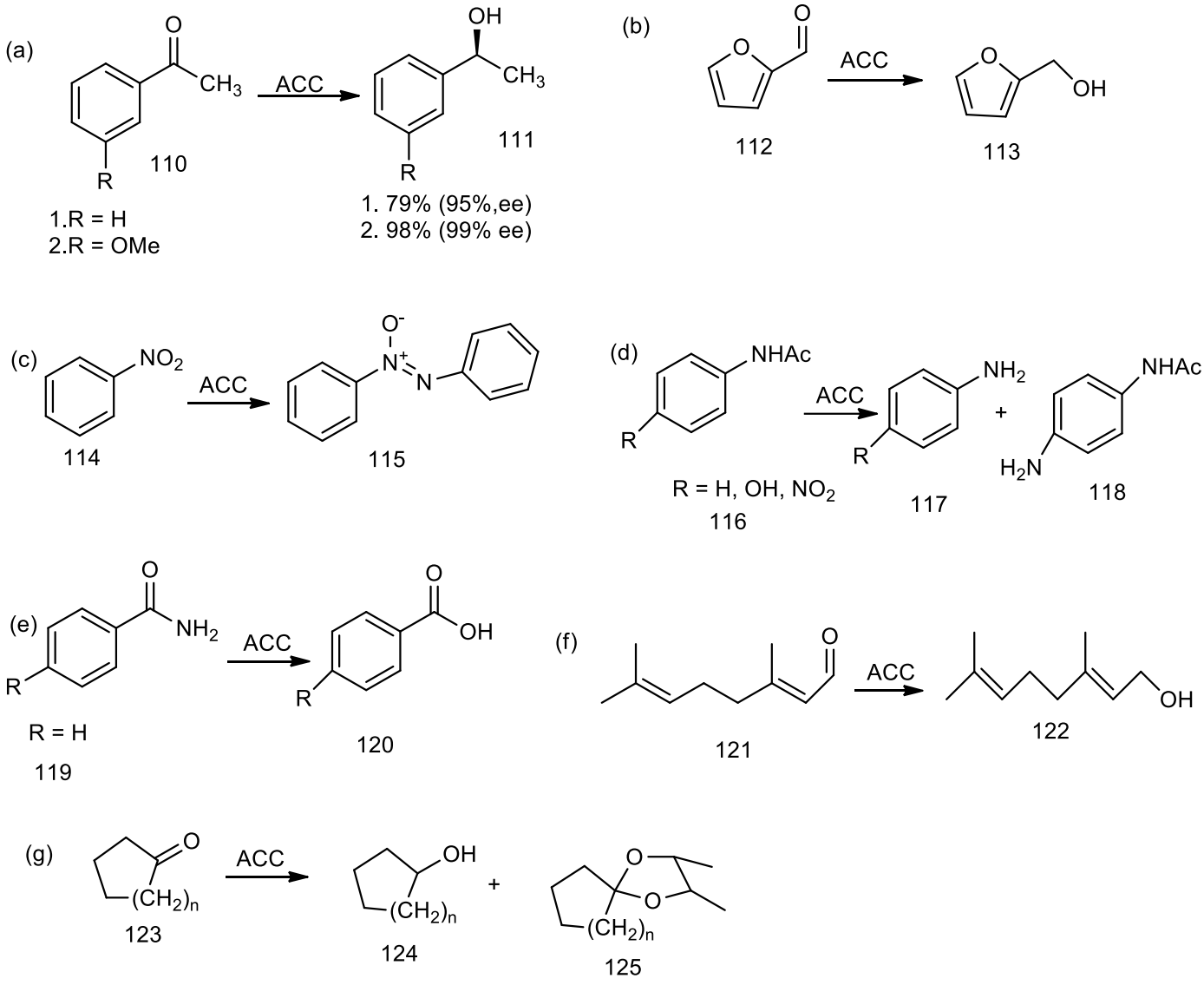
(b) oxidative cofactor recycling pattern of NAD(P)H- oxidase



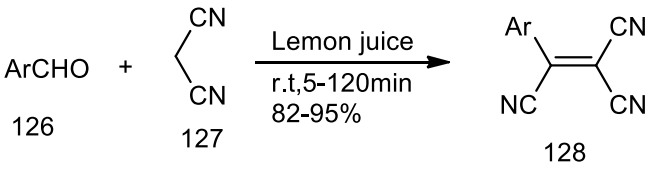
IV. FRUIT JUICE AS BIOCATALYST IN ORGANIC SYNTHESIS

Recently, attention in organic synthesis has been focused on the development of greener and eco-friendly processes which involve in the use of alternative reaction media to replace toxic and expensive catalysts as well as most volatile and hazardous solvents like benzene, toluene and methanol, commonly used in organic synthesis. The applications of aqueous extracts from different fruit juice have witnessed a rapid increase. Excellent catalytic abilities, environmentally benign character, nonhazardous and cost effectiveness are some of the reasons that have sustained interest in the use of fruit juice as biocatalysts in organic synthesis. This class of biocatalyst is now being routinely used in organic synthesis as homogeneous catalysts for various selective transformations of simple and complex molecules [32, 33].

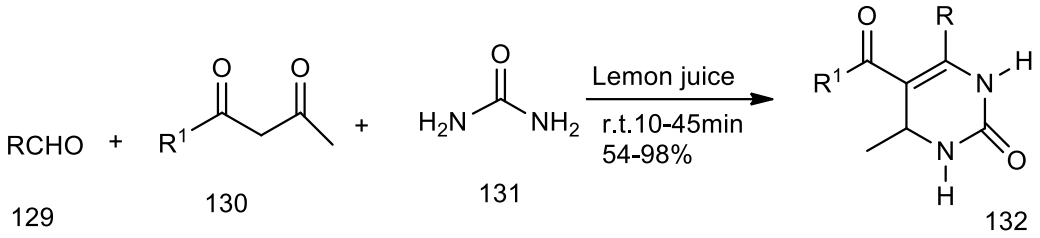
Aluísio *et al* (32) carried out series of aliphatic and aromatic aldehydes and ketones reduction using plant cell preparations from coconut juice, *Cocos nucifera*, also called ACC (água-de-coco do Ceará). The author maintained that the reduced products were obtained in excellent yields (%) and with very high enantiomeric excess. The substrates used include esters, amides, and nitrobenzene, and they yielded acids, amines and an azoxyderivative with satisfactory results as shown in the schemes below.



Rammohan Pal [33] reported on the versatile synthetic applications of fruit juice from lemon, pineapple, tamarind, *Acacia concinna*, *Sapindum trifolistus*, in organic synthesis. Lemon juice for instance was reported by the author to catalyzed reactions including Knoevenagel condensation, three-component synthesis of dihydropyrimidinones, triazoles, synthesis of schiff bases, and bis-, tris- and tetraindoles. Pineapple juice and tamarind juice were also used by the same author to catalyze the synthesis of dihydropyrimidinones and *bis*-, *tris*- and tetraindoles respectively as shown in the reactions below.

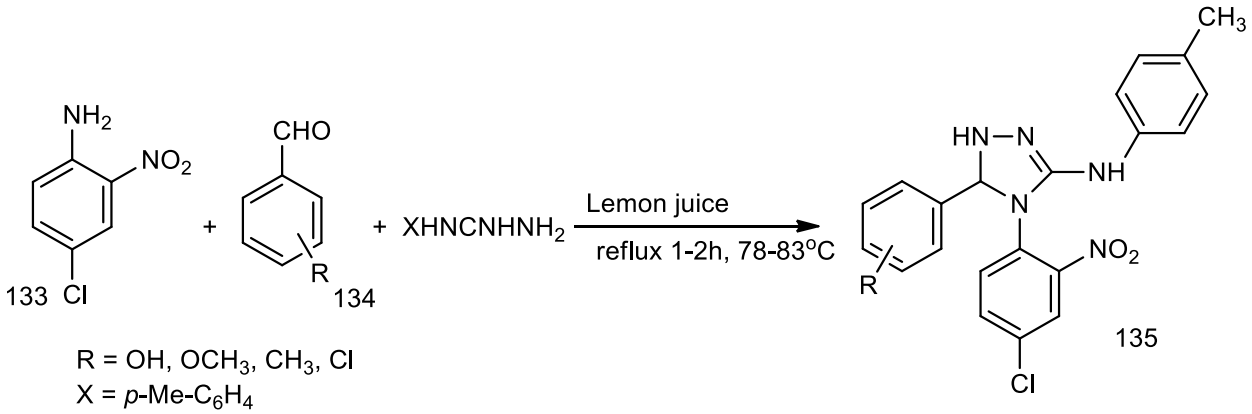


Lemon juice catalyzed Knoevenagel condesation



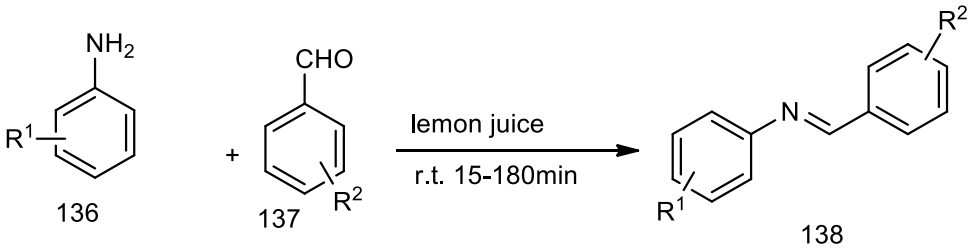
R = H, alkyl,aryl,and heteroaryl
R^1= OEt, Me

Three component synthesis of dihydropyrimidinones catalyzed.



R = OH, OCH_3, CH_3, Cl
X = *p*-Me-C_6H_4

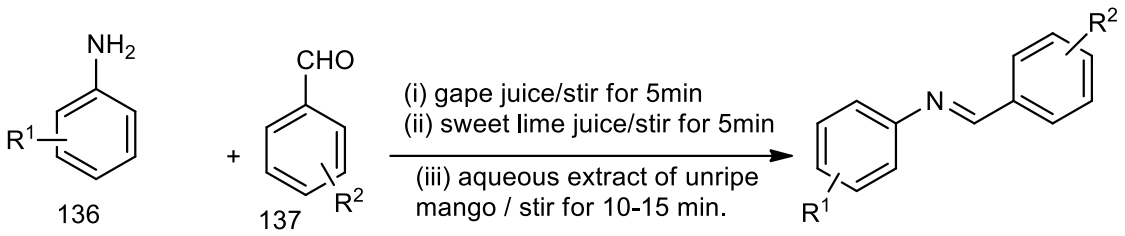
Three-component synthesis of triazole derivatives catalyzed by lemon juice



R^1 = OH, OCH_3, NO_2,
R^2 = H,Me,OMe,Br,NO_2

Lemon juice catalyzed synthesis of Schiff bases

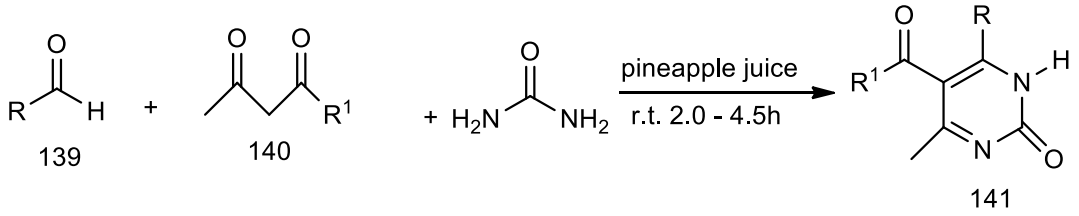
Similar reactions were also carried out by Garima Yadav and Jyoti V. Mani [34]. However, these authors made use of a mixture of grape juice, sweet lime juice and aqueous extract of unripe mango fruits to catalyze the reactions as shown in the scheme below.



R^1 = OH, OCH_3, NO_2,
R^2 = H,Me,OMe,Br,NO_2

Reaction for Schiff base synthesis in presence of acid catalysts

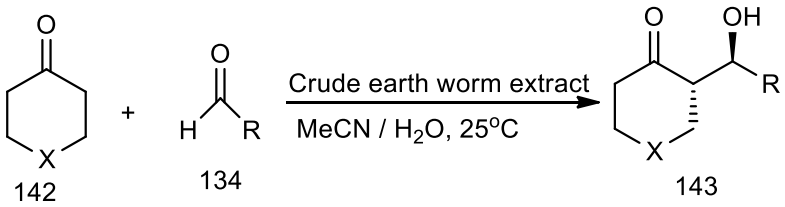
The application of pineapple juice as an efficient biocatalyst for the synthesis of dihydropyrimidinones was also reported by the above authors [33, 34]. They claimed that equimolar quantities of aldehydes, ethyl acetoacetate and urea were stirred in presence of pineapple juice at room temperature for 2-5h. This was possible due to the acidic nature of pineapple juice (pH 3.7) thus acting as a catalyst in the formation of DHPMs as shown in the reaction below.



R = H, alkyl, aryl, and heteroaryl
R^2 = OEt and Me

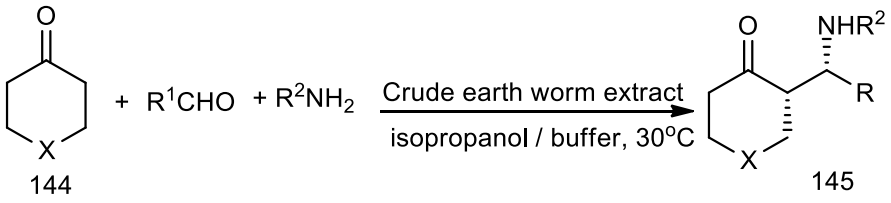
Pineapple juice catalyzed synthesis of dihydropyrimidinones

In another development, Zhi Guan *et al* [35] reported on the use of crude extract of earthworm as an eco-friendly, environmentally benign, and easily accessible biocatalyst for various organic synthesis which include the asymmetric direct aldol and Mannich reactions, Henry and Biginelli reactions, direct three-component aza-Diels-Alder reactions for the synthesis of isoquinuclidines, and domino reactions for the synthesis of coumarins. The authors maintained that these reactions have never before seen in nature, and moderate to good enantioselectivities in aldol and Mannich reactions were obtained with this earthworm catalyst. They also claimed that the products can be obtained in preparative useful yields, and the procedure does not require any additional cofactors or special equipment. The schemes below illustrate some of the transformations achieved by Zhi Guan and coworkers using crude extract of earthworms.



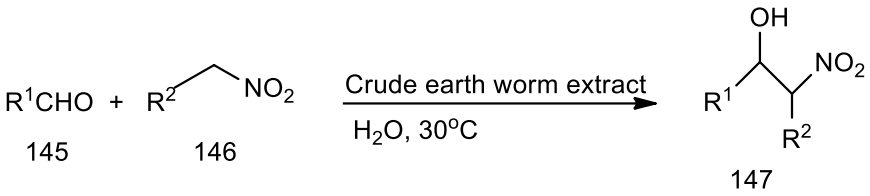
R = 4-$NO_2C_6H_4$, 4-ClC_6H_4, 4-$MeOC_6H_4$
X= CH_2 , S

The crude earthworm extract catalysed direct asymmetric aldol reactions.



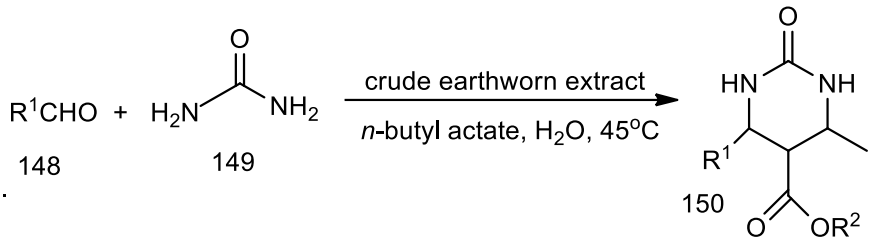
R^1 = 4-$NO_2C_6H_4$, 4-ClC_6H_4
R^2 = 3-BrC_6H_4, 4-ClC_6H_4, 4-MeC_6H_4
X= CH_2, S

The crude earthworm extract catalysed direct asymmetric Mannich reactions.



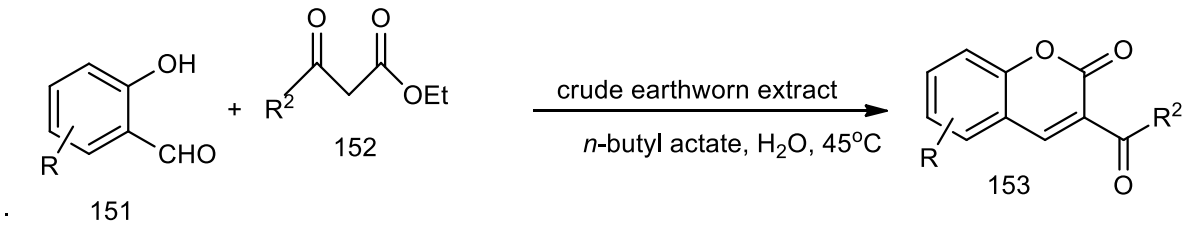
R^1 = 3-CNC_6H_4
R^2 = H, Me, Et

The crude earthworm extract catalysed Henry reactions.



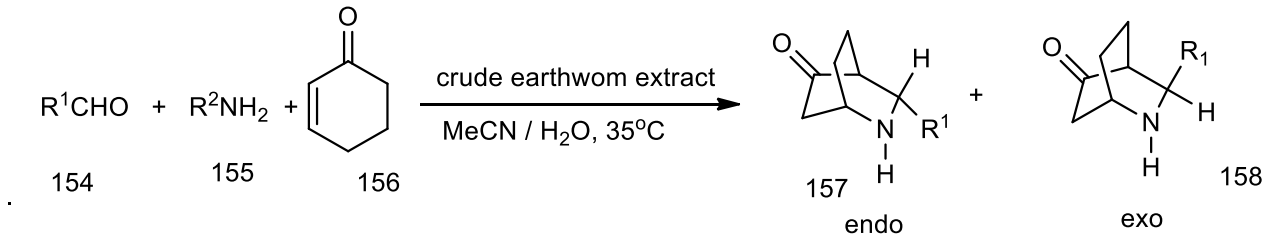
R^1 = 3-MeC_6H_4, $NO_2C_6H_4$, C_6H_5
R^2 = Me, Et

The crude earthworm extract catalysed Biginelli reactions



R^1 = H, 5-NO_2, 5-Cl, 5-OMe, 1-naphth
R^2 = Ph, Et, Me

The crude earthworm extract catalysed domino reactions for the synthesis of coumarin derivatives.



R^1 = 3-FC_6H_4, 4-FC_6H_4, 3-ClC_6H_4, 4ClC_6H_4
R^2 = 4-MeC_6H_4, C_6H_5

The crude earthworm extract catalysed aza-Diels-Alder reactions.

V. CONCLUSION

In conclusion, due to impressive interactions between biology, chemistry, and engineering in recent decades' enzyme catalysis has become an attractive synthetic tool in organic chemistry, thus

complementing existing classic chemical and chemocatalytic approaches. Today a broad range of organic reactions such as redox reactions, hydrolytic reactions, transfer reactions, carbon–carbon bond formation etc can be carried out very efficiently by means of biocatalysts. Furthermore, biocatalysis has developed towards a broadly applied production technology in the chemical industry, in particular in the fields of fine chemicals and pharmaceuticals. In future, it is expected that many more biocatalytic reactions types running in a highly efficient manner, suitable for industrial-scale applications will be achieved too. It is expected that besides optimization of known biocatalytic reactions expansion towards new type of reactions types will be possible by means of protein engineering techniques and other natural biocatalysts usage. Another challenge in the future will be the further implementation of biocatalytic reactions into multistep synthesis of (chiral) building blocks such as pharmaceuticals. This field consists of the development of alternative retrosynthetic approaches to drugs based on biocatalytic key steps as well as the development of multistep one-pot syntheses with biocatalytic reactions. Furthermore, the use of fruit juice as biocatalysts is an interesting area that is also expected to gain serious attention by synthetic chemists.

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