1

INTRODUCTION

Throughout human history, natural products, compounds that are derived from natural sources such as plants, animals, or microorganisms, have played a very important role in health care and prevention of diseases. For example, some of the first records on the use of natural products in medicine were written in cuneiform in Mesopotamia on clay tablets and date to approximately 2600 BC; Chinese herb guides document the use of herbaceous plants as far back in time as 2000 BC; Egyptians have been found to have documented the uses of various herbs in 1500 BC.

However, it's only in the nineteenth century that scientists isolated active components from various medicinal plants. The first commercial pure natural product introduced for therapeutic use is considered to be the narcotic morphine, in 1826. Natural products still play a very important role in modern medicine; in fact, they are increasingly the primary sources in drug discovery.

The pathways for generally modifying and synthesizing carbohydrates, proteins, fats, and nucleic acids are found to be essentially the same in all organisms, except for minor variations. Metabolism encompasses a wide variety of reactions for building molecules that are necessary to the life of the organism and for disruption of others for energy or secondary metabolites.

Primary metabolites are compounds that are essential for an organism's survival, growth, and replication. Secondary metabolites, such as alkaloids, glycosides, flavonoids, and so on, which are biosynthetically derived from primary

Biosynthesis of Heterocycles: From Isolation to Gene Cluster, First Edition.
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metabolites, are substances that are often present only in certain types of specialized cells, and are not directly involved in the normal growth, development, or reproduction of an organism. They represent chemical adaptations to environmental stresses, or serve as defensive, protective, or offensive chemicals against microorganisms, insects, and higher herbivorous predators. They are sometimes considered as waste or secretory products of metabolism and are of pharmaceutical importance.

The building blocks for secondary metabolites are derived from primary metabolism. In fact, the biosynthesis of secondary metabolites is derived from the fundamental processes of photosynthesis, glycolysis, and the Krebs cycle to afford biosynthetic intermediates, which, ultimately, results in the formation of secondary metabolites also known as *natural products*. The most important building blocks employed in the biosynthesis of secondary metabolites are those derived from the intermediates: acetyl-coenzyme A (acetyl-CoA), shikimic acid, mevalonic acid, and 1-deoxyxylulose-5-phosphate (Figure 1.1).

Acetyl-CoA is formed by the oxidative decarboxylation of the glycolytic pathway product pyruvic acid. Shikimic acid is produced from a combination of phosphoenolpyruvate, a glycolytic pathway intermediate, and erythrose 4-phosphate, obtained from the pentose phosphate pathway. Mevalonic acid is itself formed from three molecules of acetyl-CoA. Deoxyxylulose phosphate originates from a combination of pyruvic acid and glyceraldehyde-3-phosphate (GAP). Moreover, other building blocks based on amino acids (e.g., phenylalanine, tyrosine, tryptophan, lysine, ornithine) (Figure 1.2) are frequently employed in natural product synthesis (e.g., proteins, alkaloids, antibiotics). Though the number of building blocks is limited, the number of novel secondary metabolites formed is infinite.

Biosynthesis of secondary metabolites involves numerous different mechanisms and reactions that are enzymatically catalyzed using several common mechanisms such as acylation, alkylation, decarboxylation, phosphorylation, hydride transfer, oxidation, elimination, reduction, condensation, rearrangement, and so on. The biosynthetic pathway may undergo changes due to natural causes (e.g., viruses or environmental changes) or unnatural causes (e.g., chemical or radiation) in an attempt to adapt or provide long life to the organism.

The elucidation of the biosynthetic pathway for the production of various metabolites has been extensively examined through the use of techniques that use isotopic labeling (stable isotopes and radioactive isotopes). Initially, radiolabeled precursors were introduced into plants and the resultant radioactive compounds were chemically degraded to identify the positions of the label. As the development of analytical instrumentation advanced, the isotopically labeled natural products were analyzed by mass spectrometry (MS) and nuclear magnetic resonance (NMR) spectroscopy instead of chemical degradation.

The biosynthesis of each secondary metabolite is catalyzed by a number of enzymes, usually encoded by a gene cluster. The disclosure of biosynthetic gene clusters has great potential for the identification of entire biosynthetic pathways for bioactive compounds of pharmaceutical interest.

Genome sequence analysis provides a source of the information necessary for predicting the biosynthesis pathways for secondary metabolites because the sequence

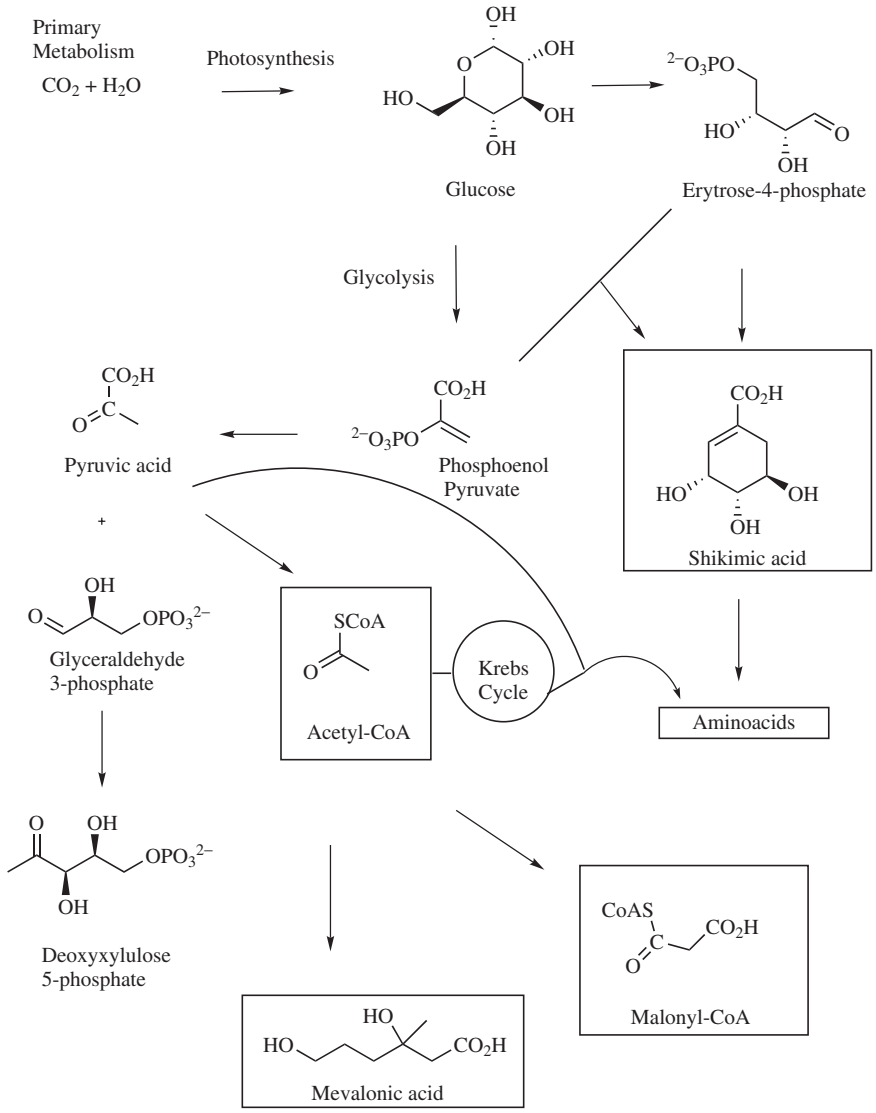


Figure 1.1 Building blocks employed in the biosynthesis of secondary metabolites.

analysis could reveal all the enzymes specific to each organism from their genes coded on the genome.

However, the gene information is not always described in a comprehensive manner and the related information is not always integrated. The database BIoSynthesis clusters CUrated and InTegrated (DoBISCUIT) integrates the latest literature information and provides standardized gene/module/domain descriptions related to the gene clusters [1].

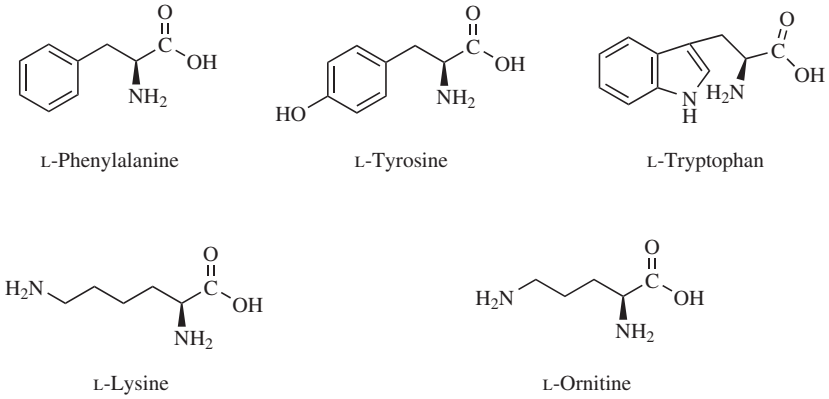


Figure 1.2 Building blocks based on amino acids.

The explanation of the biosynthetic pathway may also be possible through molecular biology techniques that use mutants. The use of tandem analytical instrumentation (e.g., GC/MS (gas chromatography/mass spectrometry), NMR/MS, LC/MS (liquid chromatography/mass spectrometry)) has improved the identifications of primary and secondary metabolites.

1.1 NATURAL PRODUCTS: PRIMARY AND SECONDARY METABOLITES

Primary metabolites can originate from fundamental processes: photosynthesis, glycolysis, and the citric acid cycle (Krebs cycle). They represent biosynthetic intermediates useful as building blocks for the synthesis of secondary metabolites. The latter can be synthesized through a combination of various building blocks (Figure 1.3):

1. a single carbon atom (C_1), usually in the form of a methyl group, obtained from L-methionine;
2. a two-carbon unit (C_2), an acetyl group, derived from acetyl-CoA or from the more active malonyl-CoA;
3. a branched chain (C_5), the isoprene moiety, formed from mevalonic acid or methylerythritol phosphate;
4. a phenylpropyl moiety (C_6C_3) and a (C_6C_2N) fragment, both originating from L-phenylalanine or L-tyrosine;
5. an indole C_2N group, obtained from L-tryptophan;
6. C_4N and C_5N portions, generated from L-ornithine and L-lysine, respectively.

Secondary metabolites can be synthesized by combining several building blocks of the same type, or by using a mixture of different building blocks.

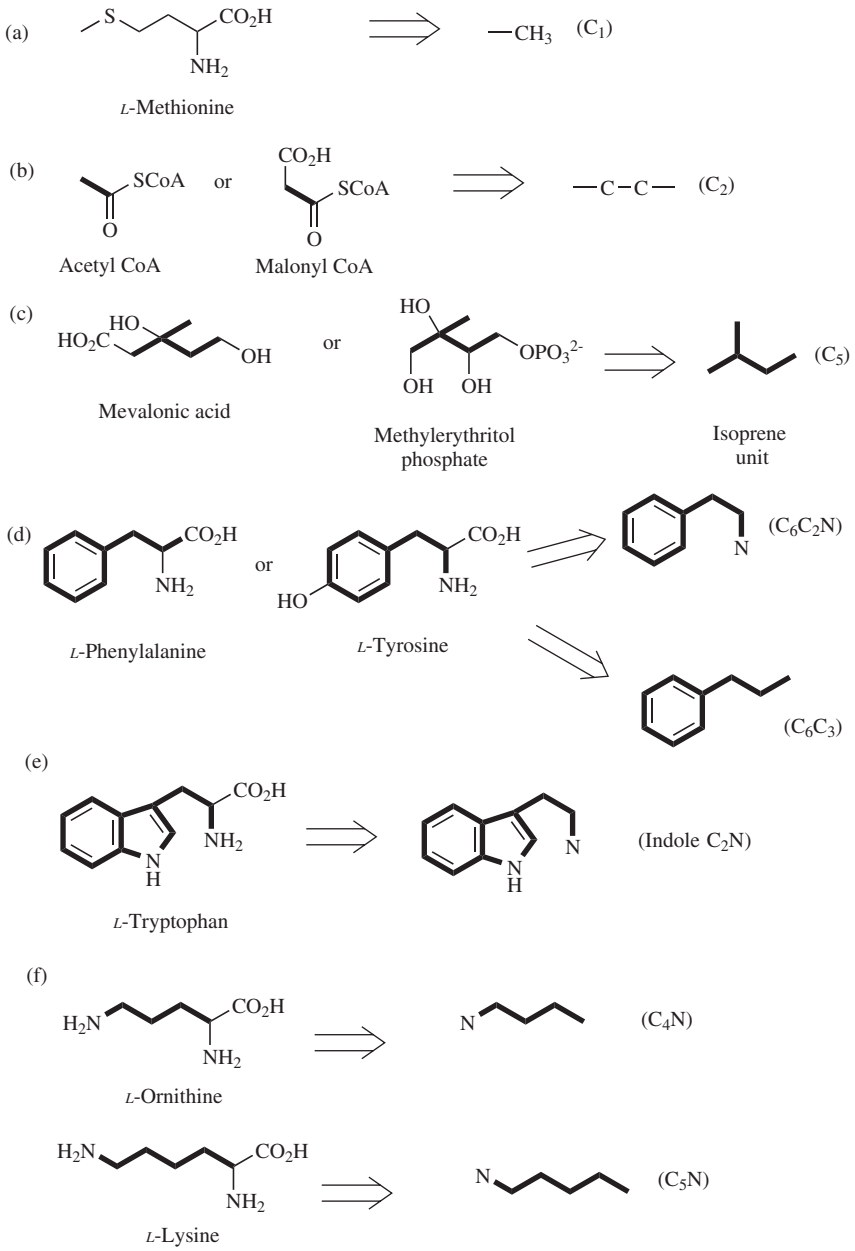


Figure 1.3 (a–f) Biosynthetic intermediates useful as building blocks for the synthesis of secondary metabolites.

Some examples of secondary metabolites are antibiotics, alkaloids, anthraquinones, coumarines, flavonoids, xanthones, and terpenoids.

1.2 COMMON REACTIONS IN SECONDARY METABOLITES

The building blocks used in the biosynthesis of secondary products are assembled through biochemical reactions and catalyzed by enzymes, including alkylation reactions (nucleophilic substitutions and electrophilic additions); Wagner–Meerwein rearrangements; aldol and Claisen reactions; Schiff base (SB) formation and Mannich reactions; transaminations, decarboxylations, oxidation, and reduction reactions (hydrogenation/dehydrogenation reactions); monooxygenase and dioxygenase reactions; Baeyer–Villiger reactions; oxidative deamination reactions; dehalogenation–halogenation reactions; and glycosylations.

1.2.1 Alkylations

The alkylation reactions are classified, based on the character of the alkylating agent, into nucleophilic substitutions and electrophilic additions. Natural alkylating agents are *S*-adenosyl-L-methionine (SAM) and dimethylallyl diphosphate (DMAPP).

In nucleophilic substitutions, SAM is commonly used as methyl donor in numerous methylation reactions. The 3-amino-3-carboxypropyl (acp) group of SAM can also be transferred to different acceptor molecules. SAM-dependent acp-transfer reactions are relatively rare compared to methyl-transfer ones.

The positively charged sulfonium ion in SAM makes the three carbon atoms that are bonded to the sulfur atom prone to attack by nucleophiles. When the alkyl acceptor is a heteroatom (most commonly O, N), the methyl- or the acp-transfer reactions occur via simple nucleophilic mechanism (S_N2): *O*-methyl or *O*-acp and *N*-methyl or *N*-acp linkages may be generated using hydroxyl and amino functions as nucleophiles (Figure 1.4). Some examples of *O*-methylation in the presence of SAM as the donor methyl group are depicted in Figure 1.5.

In the biosynthesis pathway for 3-alkyl-2-methoxypyrazines (MPs) – an important group of natural flavor constituents of some foods and raw vegetables including grapes – the methylation of 3-alkyl-2-hydroxypyrazines (HPs) is mediated by the *Vitis vinifera* genes *O*-methyltransferase proteins (VvOMTs). These genes encode the SAM-dependent *O*-methyltransferases, which have the ability to methylate HPs, which are the putative final intermediates in MP production. As a products of this reaction, 3-alkyl-2-MP and *S*-adenosylhomocysteine are generated (Figure 1.5a) [2].

Mycophenolic acid (MPA) is being used as an immunosuppressant in patients undergoing kidney, heart, and liver transplants. The final step in the biosynthesis of MPA involves the transfer of a methyl group from SAM to the demethylmycophenolic acid (Figure 1.5b) [3].

The last step of the biosynthetic pathway of Khellin and Visnagin (coronary vasodilators and spasmolytic agents) involves a methylation of 5,7-dihydroxy- and

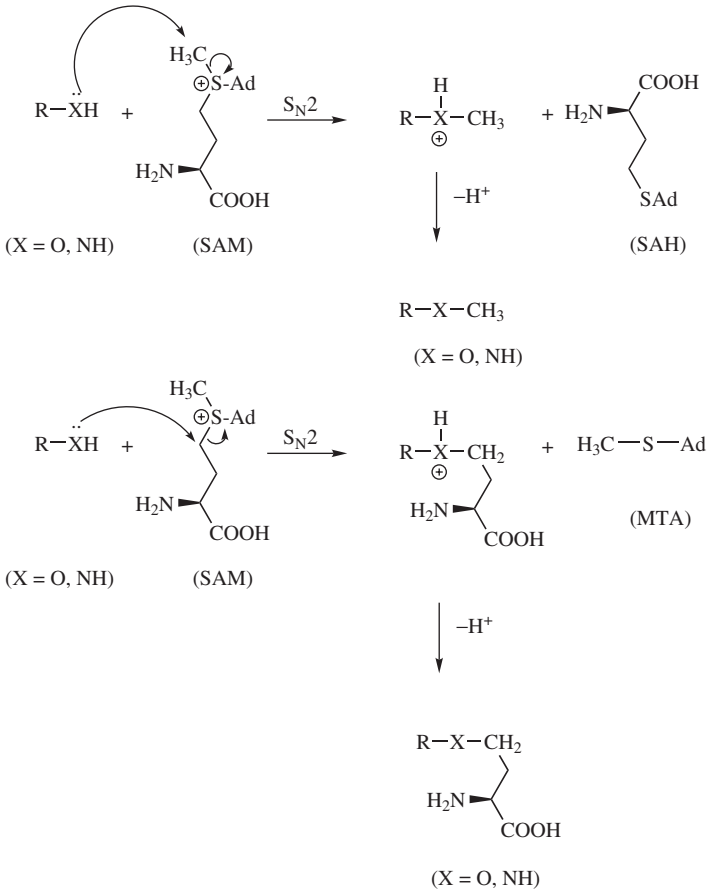


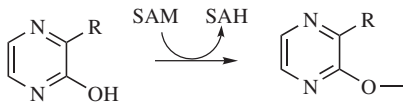
Figure 1.4 *O*- and *N*-alkylation using SAM.

5-hydroxy-furochromone, respectively, in presence of SAM as a methyl donor group. Also, the furocoumarine xanthotoxol generates xanthoxin as a result of SAM methylation (Figure 1.5c) [4].

The alkaloid anhalonine was generated by methylation of the corresponding 1,2,3,4-tetrahydro-6,7-dimetoxy-8-hydroxy-1-methylisoquinoline (anhalonidine); Kreysigine, a benzocyclohepta-isoquinoline alkaloid, was obtained by the methylation of 1,10-dihydroxy-2,11,12-trimethoxy-4,5,6,6a,7,8-hexahydrobenzo[6,7] cyclohepta [1,2,3-*ij*]isoquinoline (floramultine alkaloid) (Figure 1.5d).

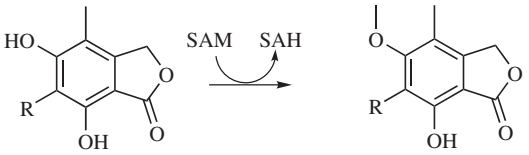
An example of *N*-methylation in presence of SAM as the methyl group donor is provided by the caffeine biosynthetic pathway involving three SAM-dependent methylation steps (Figure 1.6) [5]. The methylation reactions are catalyzed by *N*-methyltransferases (CaXMT1, CaMXMT1, and CaDXMT1), which, respectively, convert xanthosine into 7-methylxanthosine, 7-methylxanthine into

(a) 3-Alkyl-2-hydroxypyrazines



R = Isopropyl, isobuthyl

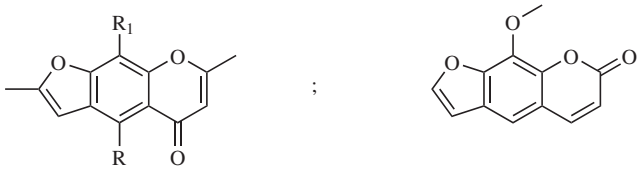
(b) Demethylmycophenolic acid



Mycophenolic acid

R = CH_2-CH=CH(CH_3)-(CH_2)$_2$-COOH

(c) Furocoumarins

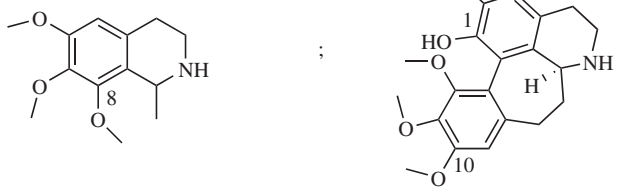


R = R_1 = OMe Khellin

R = OMe; R_1 = H Visnagin

Xanthotoxin

(d) Alkaloids



Anhalonine

Kreysigine

Figure 1.5 Examples of SAM *O*-methylations. (a) 3-Alkyl-2-hydroxypyrazine, (b) mycophenolic acid, (c) furocoumarins (khellin, visnagin, and xanthotoxin), and (d) alkaloids (anhalonine and kreysigine).

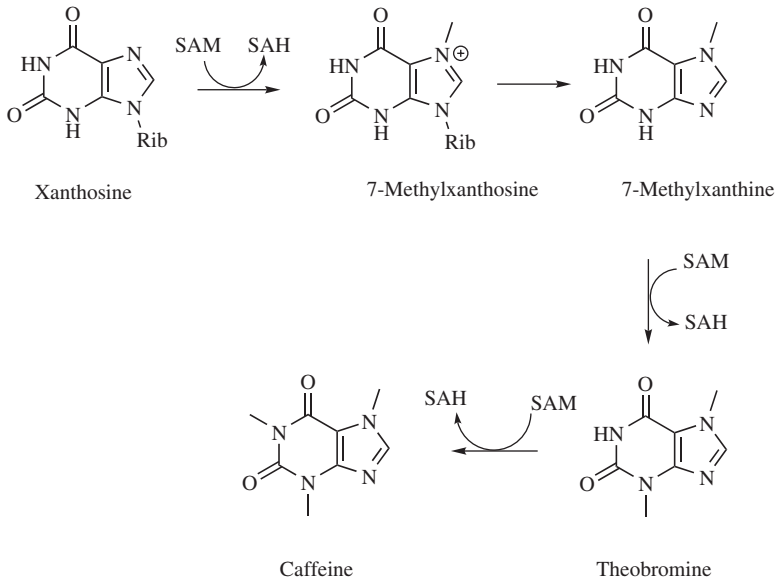


Figure 1.6 Examples of SAM *N*-methylations: biosynthesis of caffeine.

3,7-dimethylxanthine (theobromine), and the latter into 1,3,7-trimethylxanthine (caffeine). Further examples of natural compounds *N*-methylated by SAM are lophocerine and galanthamine (alkaloid derivative isolated from snowdrop *Galanthus nivalis* L.) (Figure 1.7). *N*-methylation reactions catalyzed by SAM-dependent methyltransferases are also involved in the assembly of nonribosomal peptides (NRPs) [6]. For instance, in the biosynthesis of lyngbyatoxin, an embedded SAM-dependent methyltransferase domain mediates the methylation of the free amine of the NRPS (nonribosomal peptide synthetase) [7]. A further case of *N*-methylation is provided by the biosynthesis of saframycin A (an antibiotic with antitumor activity produced by *Pseudomonas fluorescens* A2-2) [8].

An example of *O*-amino-carboxy-propylation is provided by the biosynthesis of nocardicin A (β-lactam antibiotic produced by the actinomycete *Nocardia uniformis*) (Section 4.1.5) (Figure 1.8).

Examples of *N*-amino-carboxy-propylation are mainly observed in RNA modifications, such as 3-(3-amino-3-carboxypropyl)uridine [9] or 1-methyl-3-(3-amino-3-carboxypropyl)pseudouridine [10, 11]. Another *N*-ACP-transfer reaction was also observed in the biosynthesis of 2-(3-amino-3-carboxypropyl)-isoxazolin-5-one (neurotoxic amino acid from *Lathyrus odoratus*) (Figure 1.8) [12].

In SAM-dependent alkylation reactions, when the methyl acceptors are carbon atoms, the enzymatic reaction mechanisms are more complicated and depend on the electronic properties of the acceptor molecules. The generation of C-alkyl linkages requires the formation of a nucleophilic carbon. An interesting SAM-dependent

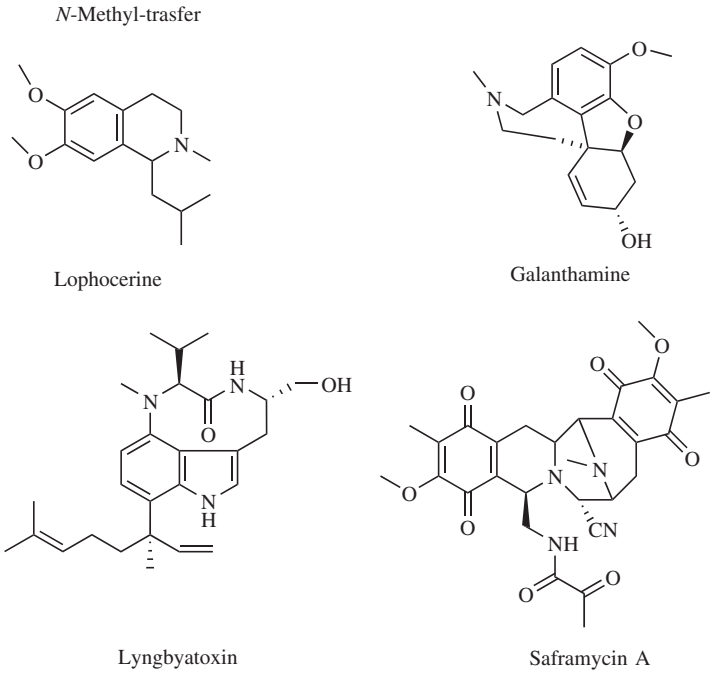


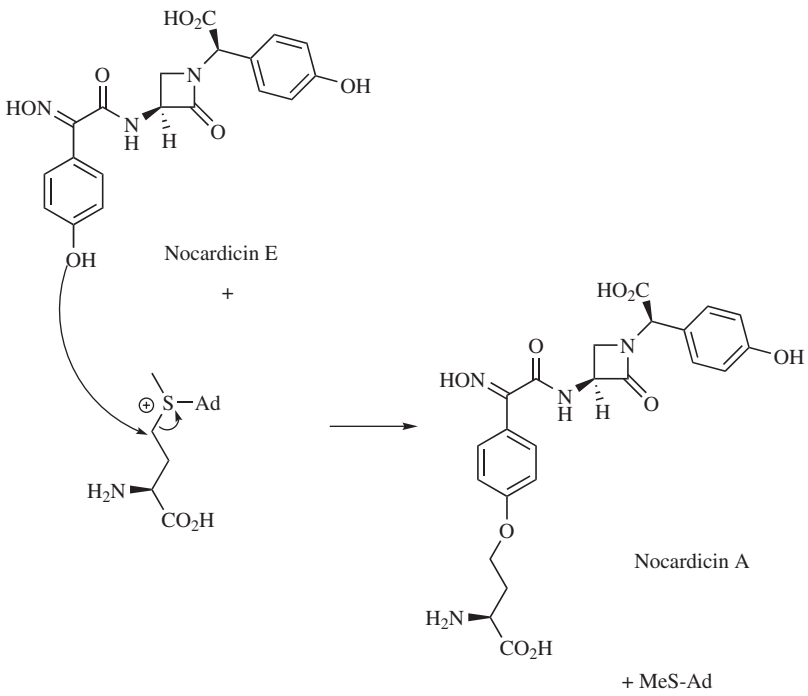
Figure 1.7 Further examples of natural compounds *N*-methylated by SAM: lophocerine, galanthamine, lyngbyatoxi, and saframycin A.

C-methylation reaction is the methylation of the C-5 position of cytosine in DNA. In this case, the carbon C-5 of cytosine cannot directly act as a nucleophile. The electron withdrawal by N-3 and the carbonyl, however, makes the C-5—C-6 double bond electron deficient and prone to attack by nucleophiles in a reaction that is similar to a Michael reaction. In DNA methyltransferases (DNMTs), this nucleophile is the thiolate from a Cys residue. The addition product is nucleophilic and reacts with SAM via an S_N2-like mechanism to capture the methyl group. The resulting intermediate then eliminates the Cys of DNMT to give the methylated cytosine product (Figure 1.9). The methylation of C-5 of cytosine is an example of converting an electron-deficient methyl acceptor to a nucleophile for the methyl-transfer reaction by addition of an active site Cys thiolate.

There are two known examples of acp transfer to carbon atoms, namely diphthamide and wybutosine (characterized by a tricyclic 1*H*-imidazo[1,2-α]purine core with a large side chain) biosyntheses.

The biosynthesis of diphthamide was proposed to involve three steps, with the first one being the acp transfer from SAM to the C-2 of the imidazole ring (Figure 1.10). The proposed reaction mechanism involves an electron transfer event from the [4FE-4S] cluster, which leads to the breaking of C—S bond and consequently generates the acp radical. The latter is added to imidazole ring, and then a hydrogen atom is eliminated to give the desired product. The formation of the acp radical is

(a) *O*-acp-transfer



(b) *N*-acp-transfer

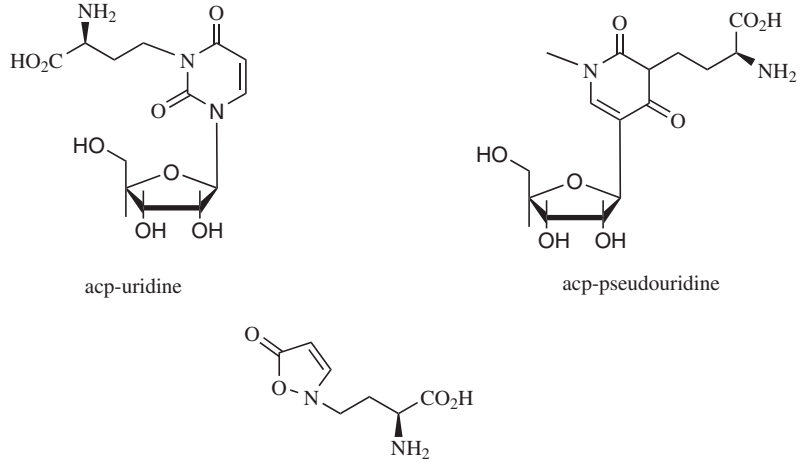


Figure 1.8 acp-transfer reactions. (a) *O*-amino-carboxy-propylation (nocardicin A) and (b) *N*-amino-carboxy-propylation (3-(3-amino-3carboxypropyl)uridine), 1-methyl-3-(3-amino-3-carboxypropyl)pseudouridine, and 2-(3-amino-3-carboxypropyl)-isoxazolin-5-one).

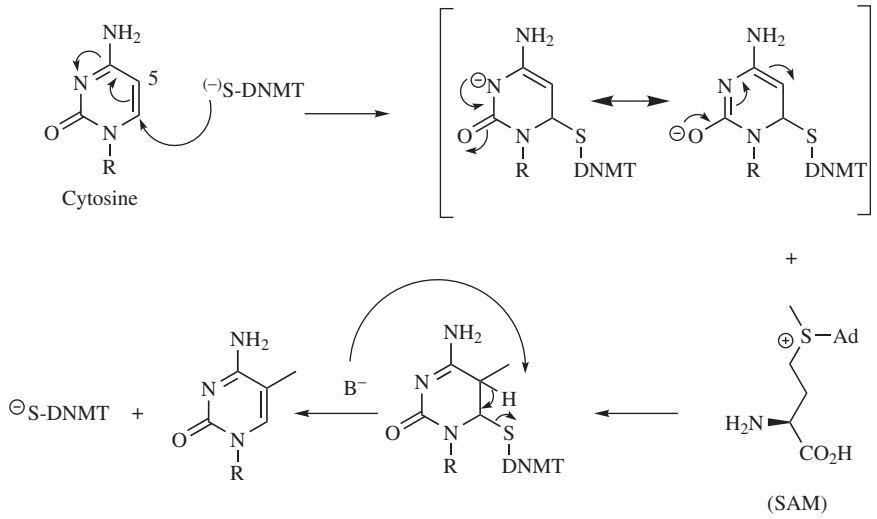


Figure 1.9 acp C-methylation of cytosine.

supported by the detection of 2-aminobutyrate and homocysteine sulfinic acid when the imidazole substrate was not present in the reaction (Figure 1.10b) [13].

In the proposed biosynthesis pathway of wybutosine, the acp-transfer step is catalyzed by Tyw2, which has similarity to methyltransferases that catalyze nucleophilic methyl-transfer reactions (Figure 1.11).

DMAPP may act as an alkylating agent (isopropene unit) via an S_N2 nucleophilic displacement in which the diphosphate is the leaving group. In some cases, DMAPP may ionize first to the resonance-stabilized allylic carbocation, and thus an S_N1 reaction occurs on the C-activated position (Figure 1.12).

The initial step in cytokinin (adenine derivatives with an isoprenoid side chain) biosynthesis is *N*-prenylation of adenosine 5-phosphate, a reaction catalyzed by adenosine phosphate-isopentenyltransferases (PTs). PTs catalyze the isopropene unit transfer reaction to an acceptor (adenosine monophosphate, AMP) which serves as a nucleophile. The latter is alkylated by DMAPP to form, by an S_N2-nucleophilic displacement reaction, a prenylated AMP and pyrophosphate (PP) as products [14, 15].

A further example of DMAPP alkylation is the *N*-prenylation of tryptophan in the biosynthesis of the cyclic peptides cyclomarin and cyclomarazine (diketopiperazine dipeptides) (Section 3.2.8) (Figure 1.13) [16].

Electrophilic additions occur frequently in the biosynthesis of steroids and terpenoids. The electrophile in such reactions is a positively charged or a positively polarized carbon atom, which often adds to an unsaturated (electron-rich) partner, usually an alkene, and leads to the formation of a saturated product. In most cases, biochemical pathways have evolved in such a way that electrophilic addition reactions

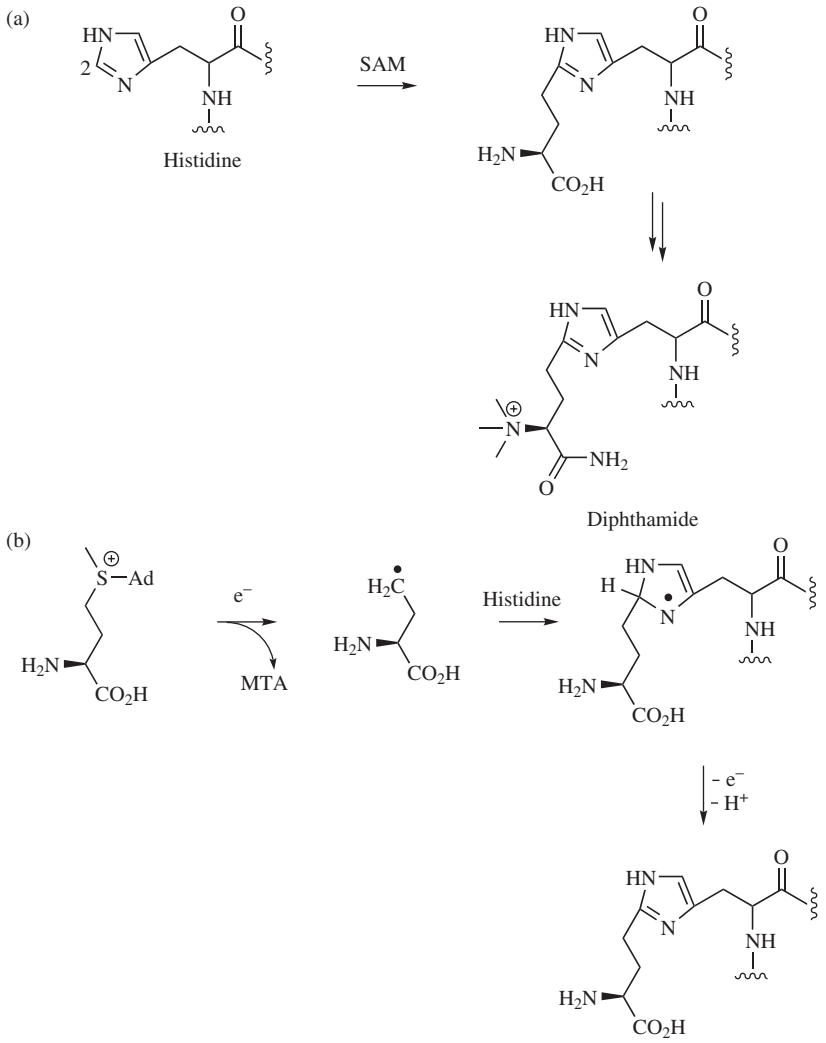


Figure 1.10 acp C-methylation in diphtamide biosynthesis: (a) methylation of hystidine and (b) the proposed mechanism.

to asymmetrical alkenes proceed through the more stable of the two possible carbocation intermediates. Methylation reactions are one of the most common selective modifications of biological macromolecules.

In Nature, methyl groups are selectively introduced into reactive aromatic rings by methyltransferases (Mtases), in particular with SAM as the cofactor. Furthermore, enzyme-catalyzed reactions are important for access to isoprenoids. SAM can act as an electrophile that transfers a methyl group to a specific nucleophilic atom.

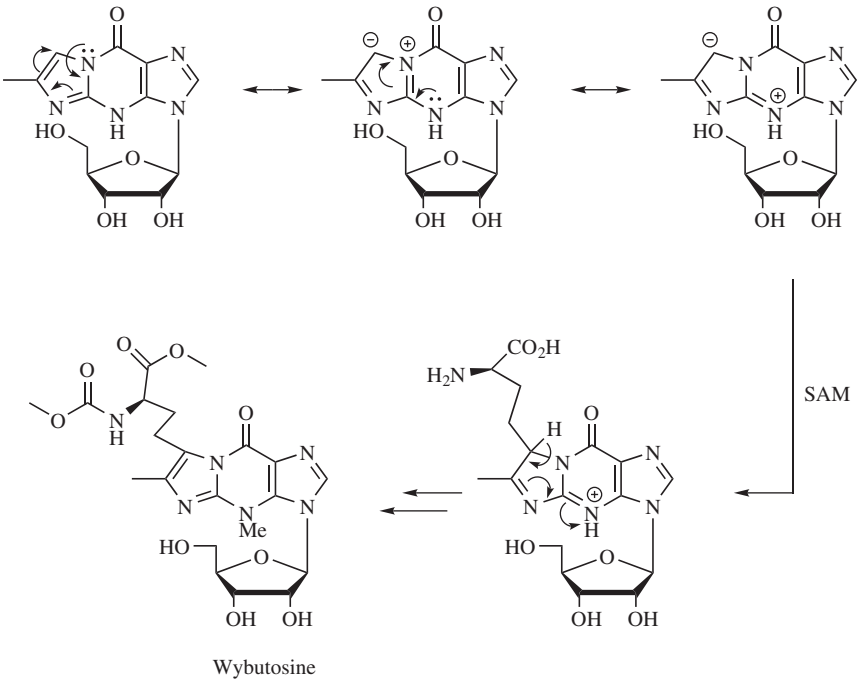


Figure 1.11 acp C-methylation in wybutosine biosynthesis.

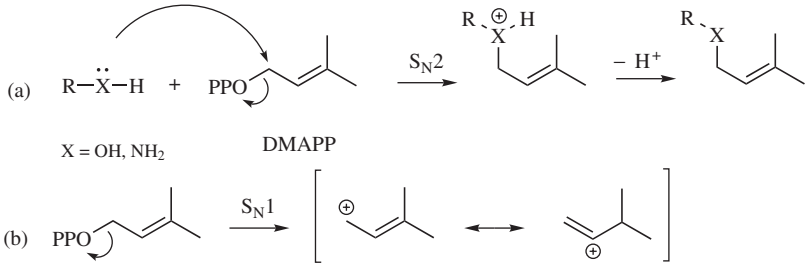
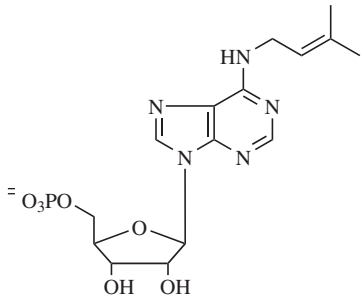


Figure 1.12 (a,b) Dimethylallyl diphosphate (DMAPP) as alkylating agent.

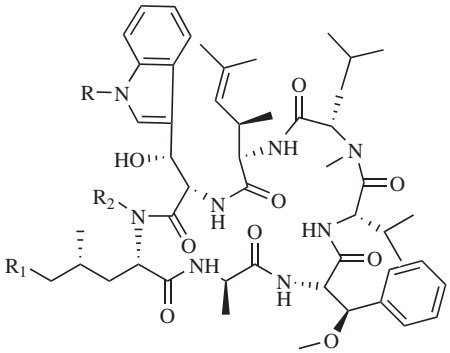
SAM alkylates alkenes by an electrophilic addition mechanism, adding a single carbon atom (C_1) and forming an intermediate carbocation (Figure 1.14a).

Also, DMAPP can be used to alkylate a nucleophile. In fact, C_5-units (used to alkylate a nucleophile) are frequently encountered as part of alkaloids (shikimate metabolites) because of "late-stage" alkylation by DMAPP.

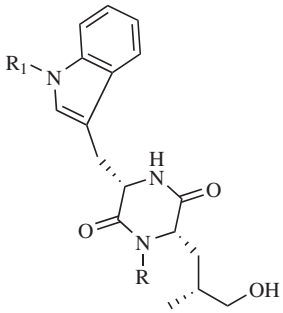
The mechanism of this reaction involves two different steps. In the first step, the DMAPP may ionize to form a resonance-stabilized allylic carbocation. The



Prenylated AMP



Cyclomarins



Cyclomarazines

Figure 1.13 Examples of DMAPP alkylation: prenylated adenosine monophosphate; diketopiperazine dipeptides (cyclomarins and cyclomarazines).

so-formed allylic carbocation then can react with an alkene (e.g., isopentenyl diphosphate, IPP). The generated carbocation loses a proton to give the corresponding alkylated product (geranyl diphosphate, GPP) (Figure 1.14b).

Some examples are represented by the steps of the reactions that lead to the biosynthesis of novobiocin (the aminocoumarin antibiotic produced by *Streptomyces spheroides* and *Streptomyces niveus*) (Figure 1.15) [17, 18], lysergic acid (ergot

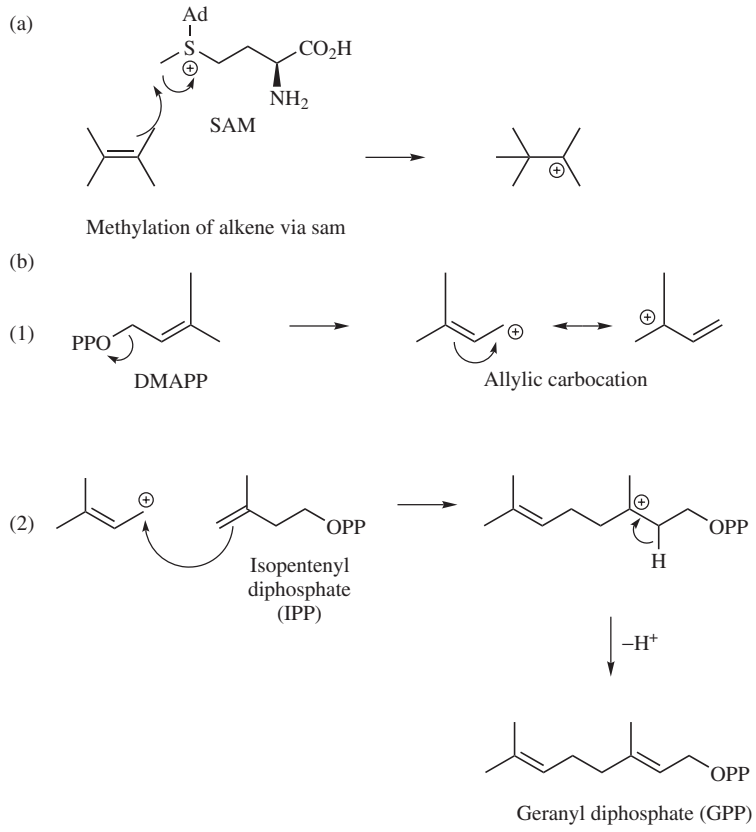


Figure 1.14 Methylation of alkene (a) via SAM and (b) via DMAPP.

alkaloid) [19], and roquefortine (an alkaloid isolated from *Penicillium roquefortine*) (Figure 1.16) [20].

1.2.2 Wagner–Meerwein Rearrangements

The migration of an alkyl group to a cationic center is known as a *Wagner–Meerwein rearrangement*. Typically, migration consists of 1,2-shifts of hydride, methyl, or alkyl groups; occasionally, 1,3 or longer shifts are encountered. These shifts are readily rationalized in terms of the generation of a more stable carbocation or relaxation of the ring strain (Figure 1.17).

Wagner–Meerwein rearrangements are prevalent in the biosynthesis of terpenoids and steroids [21]. An example can be represented by the concerted rearrangements (1,3-hydride and 1,2-methyl shift) that lead to the biosynthesis of trichodiene, an intermediate of the terpenes verrucarin A and roridin A (Section 3.2.24) (Figure 1.18).

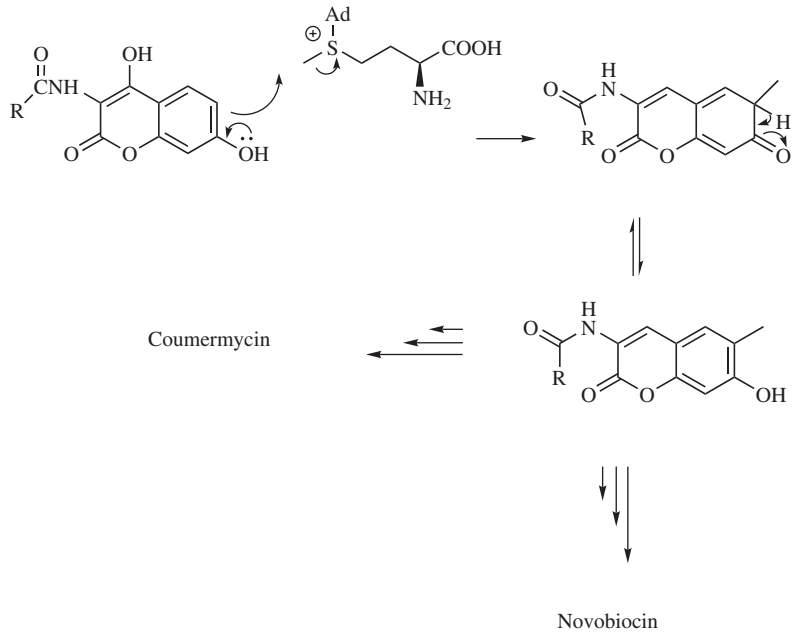


Figure 1.15 C-methylation via SAM of a coumarin intermediate leading to coumermycin and novobiocin.

1.2.3 Aldol and Claisen Reactions

Aldol and Claisen condensations are widely used in the biological world for the synthesis of new carbon–carbon bonds. In general, aldol reactions are nucleophilic carbonyl addition reactions, in which the electrophile is the carbonyl carbon of an aldehyde or ketone. One carbonyl partner with an α-hydrogen atom is converted by a base into its enolate ion. The base removes an acidic α-hydrogen, yielding a resonance-stabilized enolate ion. This enolate ion acts as a nucleophilic donor and adds to the electrophilic carbonyl group of a second aldehyde molecule to give a tetrahedral alkoxide ion intermediate. Protonation of this intermediate gives the neutral aldol product. The products of aldol reactions often undergo a subsequent elimination of water, made up of an α-hydrogen and the β-hydroxyl group. The product of this β-elimination reaction is an α,β-unsaturated aldehyde or ketone (enone).

Claisen reaction is a carbonyl condensation that occurs between two ester components and gives a β-keto ester product. The reaction has a mechanism similar to that of the aldol reaction. The difference from aldol condensation is the expulsion of an alkoxide ion from the tetrahedral intermediate of the initial Claisen adduct. This adduct is not stable and expels the ethoxide ion to give the new carbonyl compound ethyl acetoacetate (β-keto ester). Claisen products can be easily hydrolyzed and decarboxylated (Figure 1.19). There are also many examples of retro-Claisen

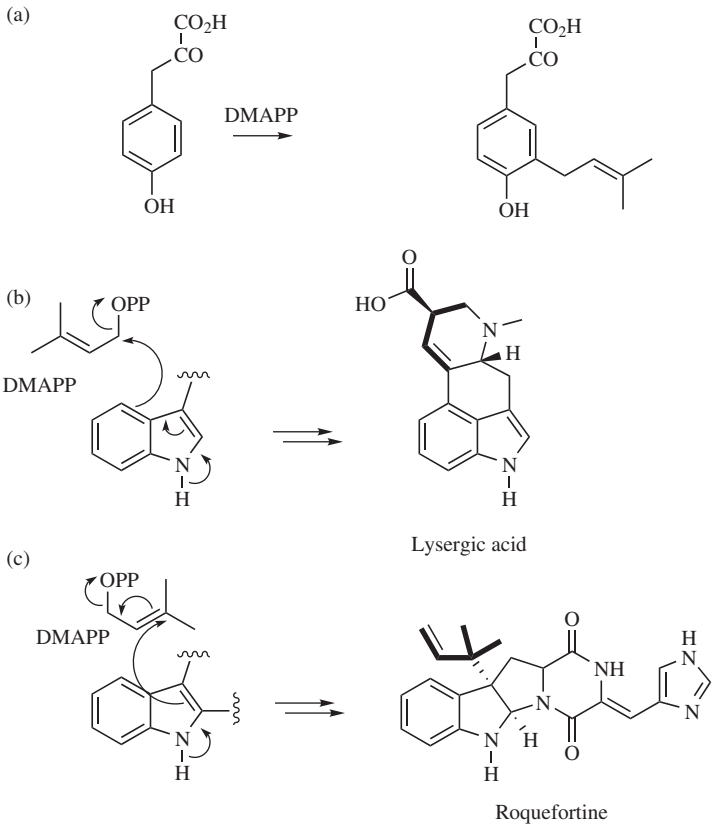


Figure 1.16 C-methylation via DMAPP. (a) Hydroxyphenyl pyruvic acid, (b) indole intermediate leading to lysergic acid, and (c) indole intermediate leading to roquefortine.

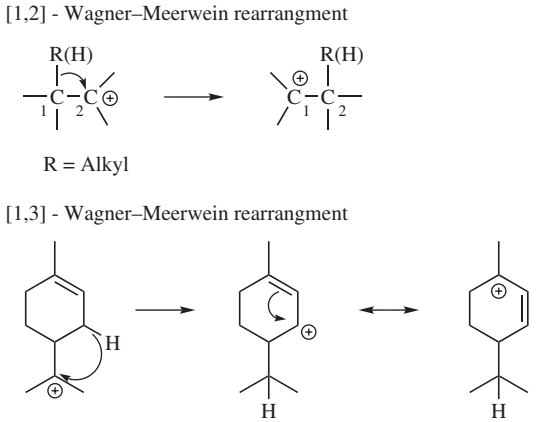


Figure 1.17 Wagner–Meerwein rearrangement mechanism.

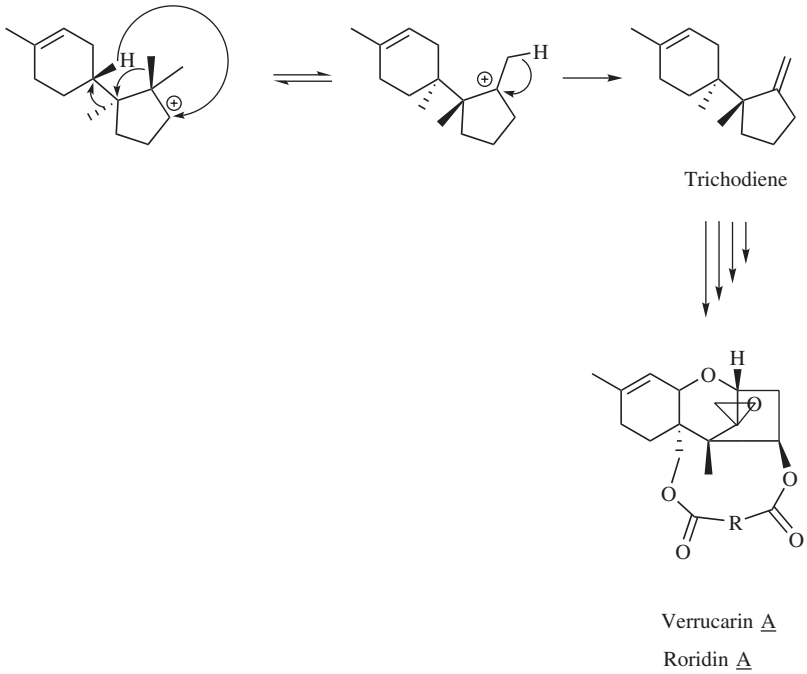


Figure 1.18 Example of concerted Wagner–Meerwein rearrangements (1,3-hydride and 1,2-methyl shift) leading to trichodiene, which is the intermediate in the biosynthesis of verrucarin A and roridin A.

cleavage reactions in biochemical pathways. In fact, since the steps in an aldol addition mechanism are readily reversible, a retro-aldol reaction can occur, converting a β-hydroxy aldehyde or ketone back to the precursors of an aldol addition.

An example of Claisen reaction is provided by the first step in the biosynthesis of cholesterol and terpenes, which starts from acetyl-CoA. Similar to the Claisen condensation, 2 equiv of acetyl-CoA couple to acetoacetyl-CoA, which represents a biological analog of acetoacetate. Following the pattern of an aldol reaction, acetoacetyl-CoA reacts with another equivalent of acetyl-CoA as a carbon nucleophile to give β-hydroxy-β-methylglutaryl-CoA (Figure 1.20).

Such a condensation is mediated by the enzyme 3-hydroxy-3-methylglutaryl-CoA synthase (HMGS). The mechanism of this catalysis is outlined in Figure 1.21. An initial *trans*-thioesterase step transfers the acetyl group of the first acetyl-CoA to an enzymatic cysteine. In the Claisen condensation phase of the reaction, the α-carbon of a second acetyl-CoA is deprotonated, forming an enolate. The enolate carbon attacks the electrophilic thioester carbon, forming a tetrahedral intermediate which quickly collapses to expel the cysteine thiol [22].

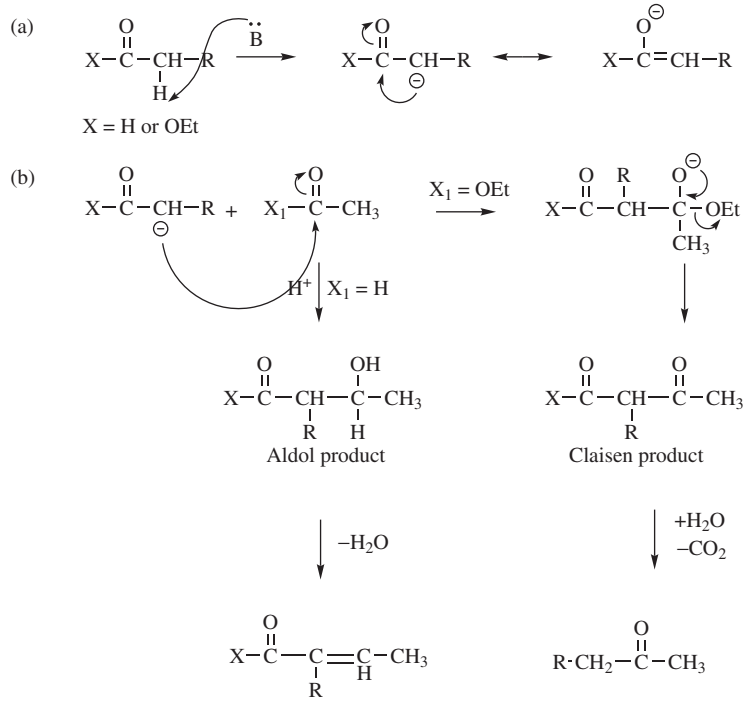


Figure 1.19 Carbonyl condensations: aldol and Claisen reactions. (a) Carbanion formation and (b) mechanisms leading to aldol and Claisen products.

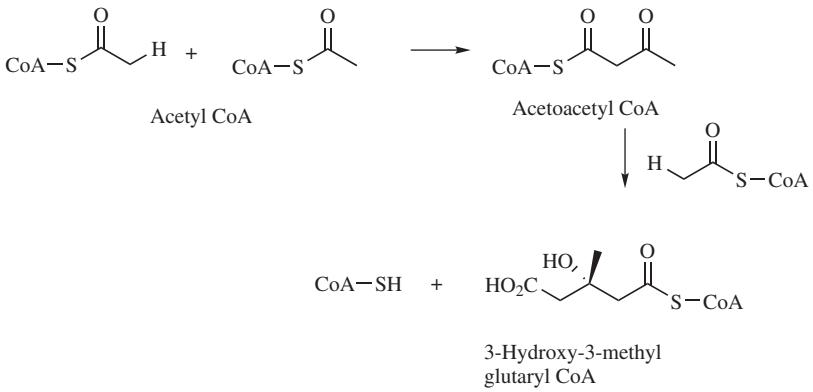


Figure 1.20 Example of Claisen reaction: synthesis of 3-hydroxy-3-methylglutaryl-CoA.

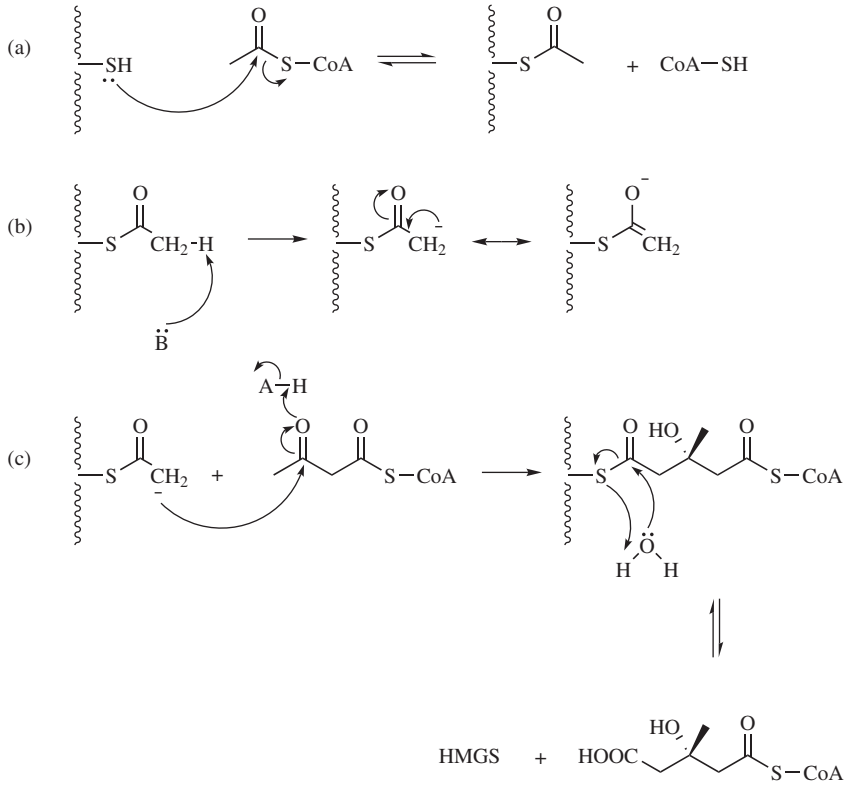


Figure 1.21 (a–c) Mechanism of the formation of 3-hydroxy-3-methylglutaryl-CoA.

Glycolysis is a fundamental pathway for the production of ATP (adenosine triphosphate) in living systems. The pathway begins with glucose and ends with two molecules of pyruvate and a net yield of two ATP molecules. Aldolase, an enzyme in glycolysis, plays a key role by dividing the fructose-1,6-bisphosphate (derived from glucose) into two compounds, each of which have three carbons, GAP and 1,3-dihydroxyacetone phosphate (DHAP). This process is essential because it provides two three-carbon units for the final stage of glycolysis, wherein the net yield of two ATP molecules per glucose is realized. The cleavage reaction catalyzed by aldolase is a net retro-aldol reaction (Figure 1.22).

The conversion of chorismate into prephenate is an example of a biologically relevant Claisen rearrangement. It is the key intermediate in the biosynthesis of aromatic amino acids (tyrosine, phenylalanine, and tryptophan) in bacteria, fungi, and higher plants (Figure 1.23) [23].

A polyketide chain is formed by the condensation between an acyl thioester intermediate and an acyl carrier protein-bound malonyl or methylmalonyl thioester previously selected and transferred to the acyl carrier protein (ACP) by the AT (acyltransferase) domain. The mechanism of this KS (ketosynthase)-catalyzed reaction,

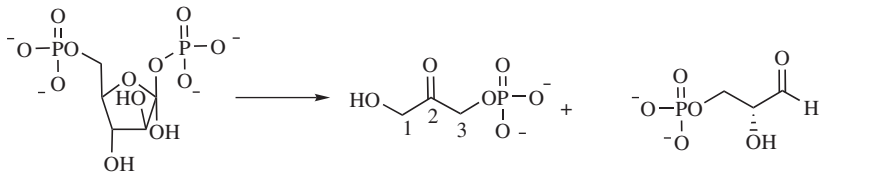


Figure 1.22 Retro-aldol reaction of β-D-fructose-1,6-biphosphate, leading to glyceraldehyde-3-phosphate and 1,3-dihydroacetonephosphate.

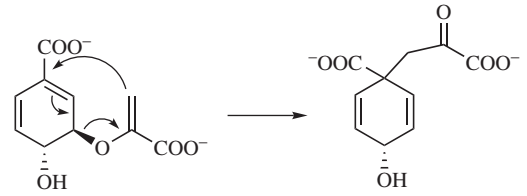


Figure 1.23 Example of Claisen rearrangement: conversion of chorismate into prephenate.

which is reminiscent of a Claisen condensation, consists of a nucleophilic addition by an α-carbanion formed by *in situ* decarboxylation to an activated thioester carbonyl, resulting in the formation of a fused β-ketoacyl thioester. Claisen-like condensation occurs with inversion of the configuration at C-2 of the malonyl thioester derivative (Figure 1.24) [24, 25].

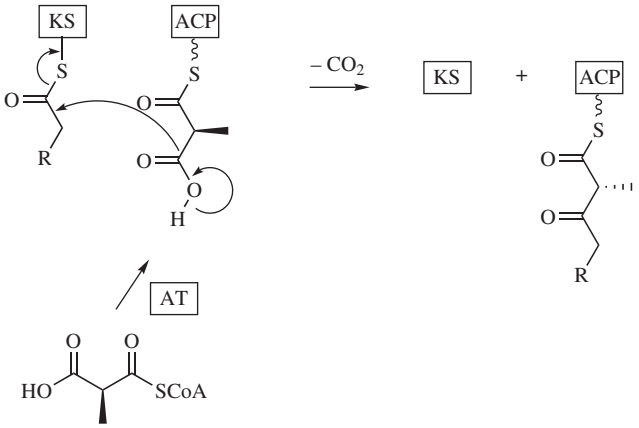


Figure 1.24 Example of Claisen condensation: formation of a polyketide chain.

1.2.4 Schiff Base Formation and Mannich Reactions

An SB contains a carbon–nitrogen double bond formed from the reaction of an amino group and an aldehyde or ketone with the elimination of water. This process is reversible and can effect the hydrolysis of imine to amine and aldehyde/ketone (Figure 1.25).

SB formation is also the first step in the Mannich reaction. In general terms, this reaction involves the formation of two covalent bonds by the condensation between an amine, a carbonyl compound, and an electronegative carbon (Figure 1.26).

The mechanism of the Mannich reaction starts with the formation of an iminium ion from the amine and formaldehyde. The compound with the carbonyl functional group tautomerizes to the enol form, and then attacks the iminium ion (Figure 1.27).

Examples of SB formation are outlined in Figure 1.28. The reaction of 4-aminobutanal and succinaldehyde yields an SB which is the intermediate of dialdehyde amine, likely a precursor of the pyrrolizidine ring system. Another example is the piperidine-2-carboxylic acid (a precursor of anabasine) obtained from α-keto-ε-aminocapronic acid.

A further intramolecular SB by condensation of 4-(methylamino)butanal leads to the *N*-methyl-Δ^1-pyrrolinium cation, which is the precursor of cocaine biosynthesis (Figure 1.28) [26, 27].

The involvement of the Mannich reaction has been proposed in many biosynthetic pathways, especially for alkaloids.

For example, a large group of alkaloids that contains the 1,2,3,4-tetrahydroisoquinoline ring system is formed by a Mannich reaction involving dopamine (derived

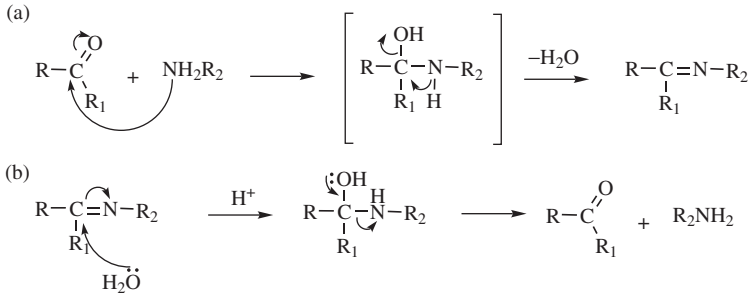


Figure 1.25 (a,b) Schiff base formation and reverse reaction.

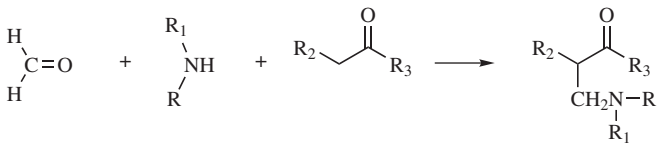


Figure 1.26 Mannich reactions.

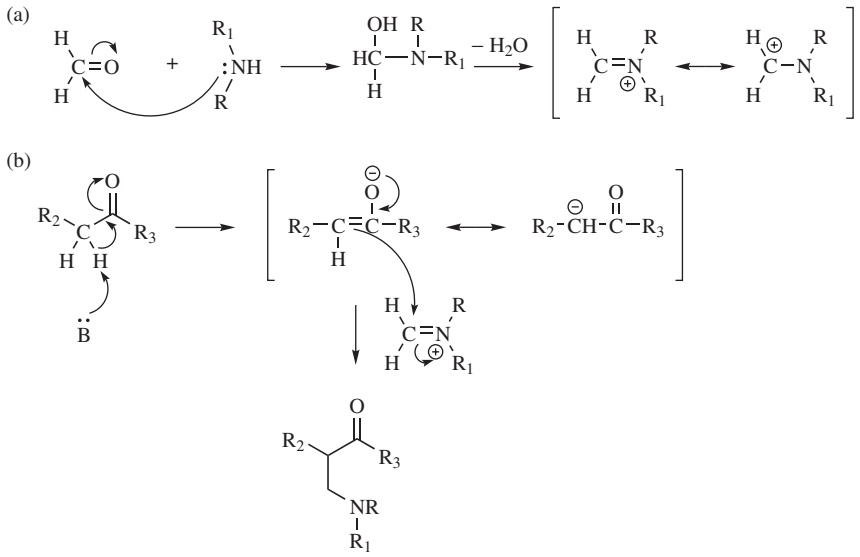


Figure 1.27 Mechanism of Mannich reactions. (a) Formation of an iminium ion from the amine and the formaldehyde and (b) tautomerization to the enol form and attack the iminium ion.

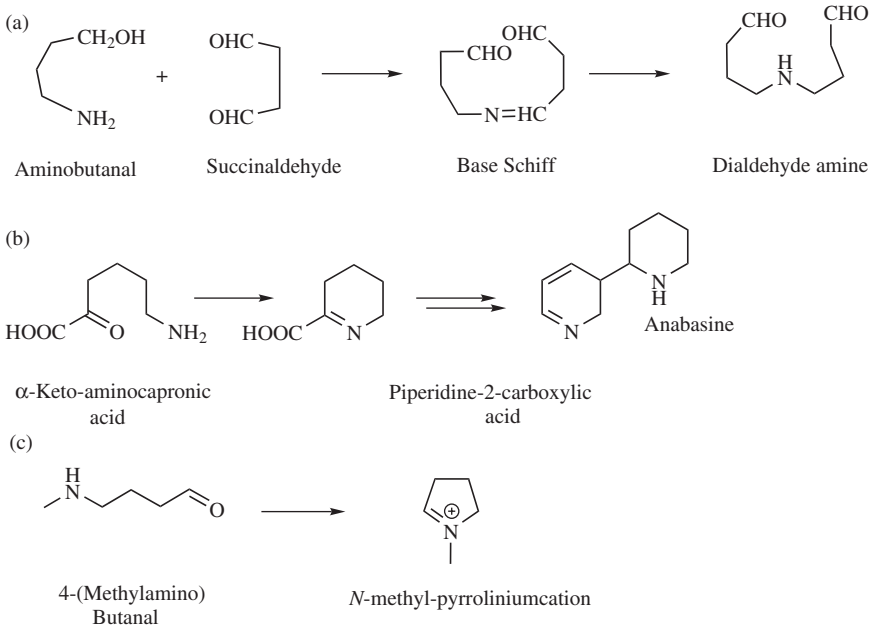


Figure 1.28 Examples of Schiff base condensations. (a) Dialdehyde amine (precursor of the pyrrolizidine ring system), (b) piperidine-2-carboxylic acid (a precursor of anabasine), and (c) *N*-methyl-Δ^1-pyrrolinium cation (precursor of cocaine).

from tyrosine via 3,4-dihydroxyphenylalanine) and a variety of carbonyl compounds (R-CO-R^1) [28, 29].

Also, a large number of indole alkaloids are formed by Mannich reactions involving tryptophan or its decarboxylation product tryptamine with various aldehydes. Both the α- and β-position of the indole nucleus are electronegative, and a Mannich reaction with tryptamine can yield a β-carboline derivative or a 3,3-spiroindolenine [30]. A further example is provided by the tropinone biosynthesis (Figure 1.29) [31].

1.2.5 Transaminations

Transamination is a reaction catalyzed by a family of enzymes called *transaminases*, which results in the exchange of an amine group of an amino acid with a ketone or a

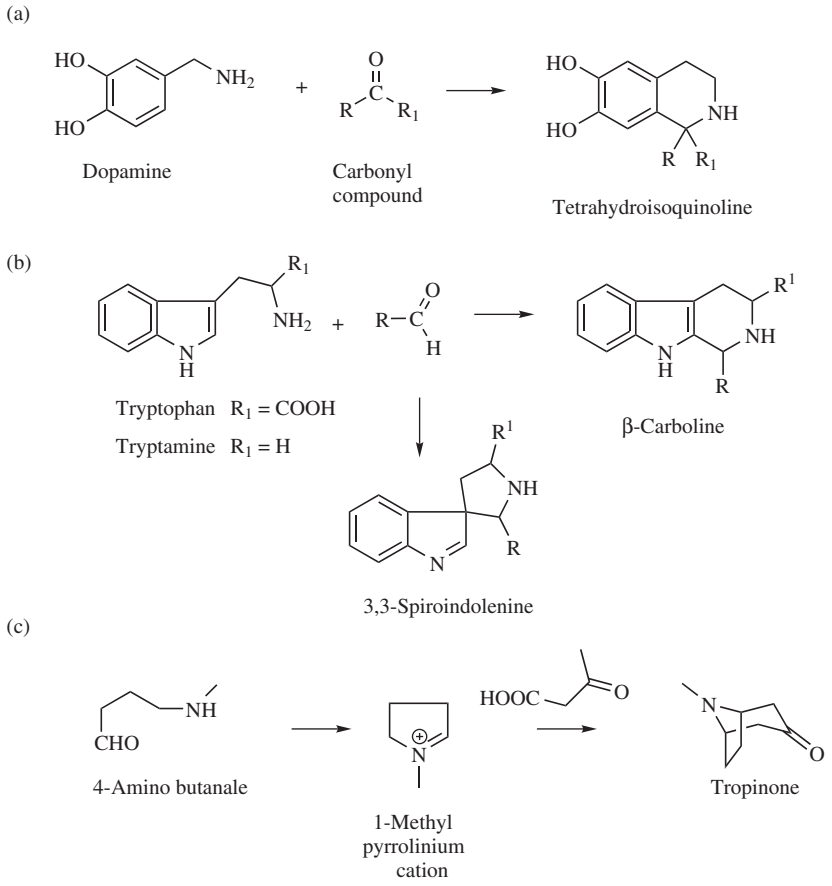


Figure 1.29 Examples of Mannich reactions. (a) Biosynthesis of alkaloids bearing the 1,2,3,4-tetrahydroisoquinoline ring, (b) biosynthesis of indole alkaloids (β-carboline, 3,3-spiroindolenine), and (c) tropinone biosynthesis.

Figure 1.30 Transamination reactions.

keto acid group of another molecule. It is analogous to a double-replacement reaction and provides the most common process for the introduction of amino acids and for the removal of the nitrogen from them (Figure 1.30).

All transamination reactions are reversible and need pyridoxal phosphate (PLP) as the coenzyme. The cofactor (PLP) is covalently bonded to the amino group of an active site lysine, forming an internal aldimine.

The transamination reaction involves three sequential steps: (i) formation of a tetrahedral intermediate with the active site lysine and the amino substrate bonded to the PLP cofactor; (ii) indirect proton transfer between the amino substrate and the lysine residue; and (iii) formation of the external aldimine after the dissociation of the lysine residue (Figure 1.31) [32, 33].

The most usual and major keto acid involved with transamination reactions is α-ketoglutaric acid ($R = (CH_2)_2COOH$), an intermediate in the citric acid cycle.

A specific example is the transamination of alanine (R = Me) to make pyruvic acid (R = Me) and glutamic acid ($R = (CH_2)_2COOH$).

Other amino acids that can be converted after several steps through transamination into pyruvic acid include serine ($R = CH_2OH$), cysteine ($R = CH_2SH$), and glycine (R = H).

1.2.6 Decarboxylations

Decarboxylation is one of the most common carbon–carbon bond-breaking processes in biological chemistry and involves the loss of a single carbon in the form of CO_2.

Decarboxylases are known for their roles in a wide variety of catabolic and anabolic pathways, including decarboxylation of α- and β-keto acids, amino acid conversions, and carbohydrate biosynthesis. Mechanistically, a decarboxylation has parallels to retro-aldol cleavage reactions (Figure 1.32).

Enzymatic decarboxylation usually utilizes an organic cofactor such as pyridoxal 5′-phosphate, biotin, flavine, pyruvoyl, and thiamine diphosphate (ThDP) in the catalytic reaction [34].

Pyridoxal 5′-phosphate (PLP)-dependent enzymes catalyze a wide range of reactions at the α-, β-, and γ-carbons of amino acids.

The decarboxylation of amino acids is a key step in the synthesis of neurotransmitter amino compounds and other physiologically important compounds. The enzyme PLP forms an internal aldimine with the α-amino group of a specific Lys residue. The obligatory first chemical step in all PLP-dependent enzymatic reactions is the formation of an SB intermediate (aldimine) between the coenzyme aldehyde and the substrate amino group. The general utility of PLP is derived from its ability to stabilize the carbanions generated adjacent to the SB in the external aldimine intermediate

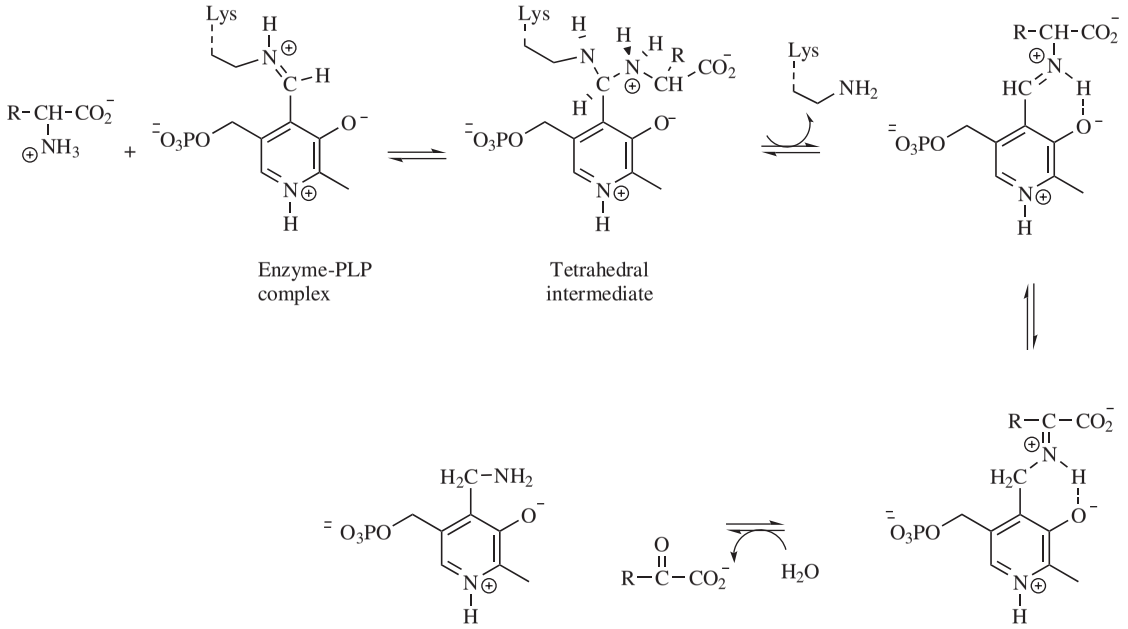


Figure 1.31 Transamination reaction mechanism.

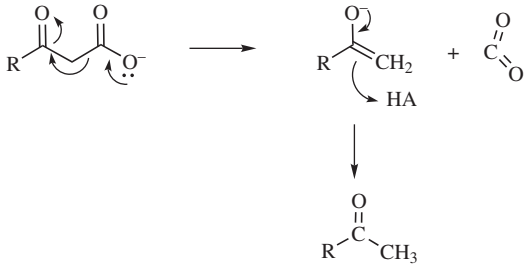


Figure 1.32 Decarboxylation reactions.

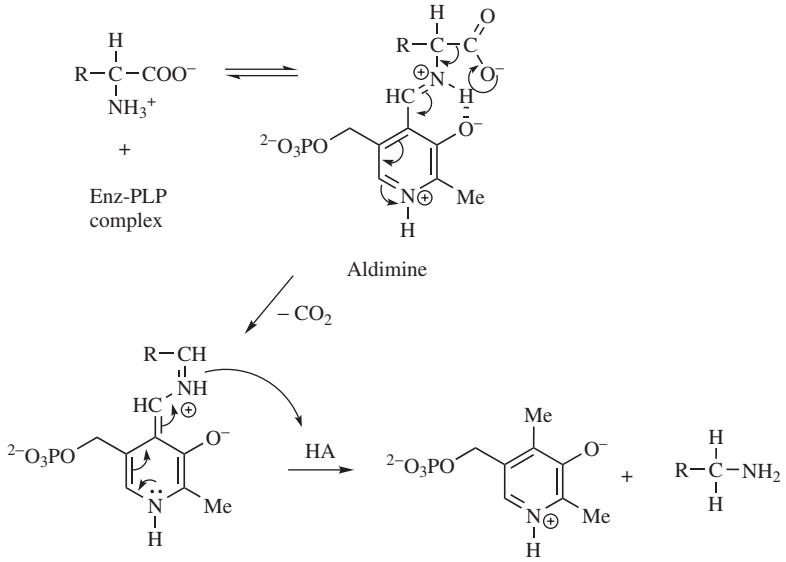


Figure 1.33 Decarboxylation of amino acids.

by delocalization of the developed negative charge into the extended π-bonded system, that is, SB and the pyridine ring. The PLP cofactor that stabilizes the developing carbanion in the transition structure leads to the loss of CO_2 (Figure 1.33).

Biotin-dependent enzymes catalyze the decarboxylation of α-keto acids and thioesters.

These enzymes are large molecules comprising three or more subunits: a biotin carboxylase subunit (α-subunit) where the enzymes pick up the carboxyl group from substrates; a decarboxylase subunit (β-subunit) where the decarboxylation takes place and the sodium ion pump is located; and a biotinyl subunit (γ-subunit) containing the specific lysine residue to which biotin covalently binds. A fourth subunit (δ-subunit) has been reported in all glutaconyl-CoA decarboxylases to anchor the α-subunit to the

membrane, while two additional subunits, δ and ϵ, are found in malonate decarboxylases and methylmalonyl-CoA decarboxylases. A zinc ion is present in the γ-subunit of the oxaloacetate decarboxylase sodium pump. The role of the metal ion is to position the oxaloacetate and to polarize the carbonyl oxygen bond of the substrate and to enhance the carboxyl transfer to biotin.

An example of biotin-dependent decarboxylase is glutaconyl-CoA decarboxylation to crotonyl-CoA. The reactions involve two distinct major steps: the first step of the reaction is a sodium-ion-independent transfer of the carboxyl group of glutaconyl-CoA to biotin. This transfer takes place in the α-subunit. The carbonyl group of the glutaconyl-CoA is polarized by hydrogen bonds to the enzyme, forming an oxyanion hole and inducing a transient decarboxylation. The hydrogen bonds between the carbonyl oxygen of biotin and the protein residues generate another oxyanion hole, which increases the acidity of the N-1 proton of biotin to promote the transfer of this proton to the dienolate anion derived from the glutaconyl-CoA. Fixation of carbon dioxide by the enzyme-bound biotin produces carboxybiotin (Figure 1.34). The second step is a decarboxylation of carboxybiotin, which takes place at the β-subunit.

Flavin-dependent decarboxylases catalyze the oxidative decarboxylation of the C-terminal peptidyl-cysteines to peptidylaminoenethils/aminoenethiolates during the biosynthesis of antibiotics. The key element of this conversion is an oxidation–reduction reaction. The CH_2SH side chain of the C-terminal cysteine residue is oxidized to a thioaldehyde or to a tautomeric enethiol with the concomitant reduction of the flavin cofactor. Decarboxylation of the thioaldehyde/enethiolate intermediate occurs spontaneously, because this step is favored by the delocalization of the negative charge of the adjacent thioaldehyde group in a manner similar to the decarboxylation of β-keto acids (Figure 1.35).

Pyruvoyl-dependent decarboxylase, through the formation of an SB with the substrate, promotes decarboxylation of amino acids. The SB intermediate is formed from the pyruvoyl moiety of the enzyme by reacting with the amino group of the amino acid substrate. In this imine intermediate, the acyl-carbonyl group of the pyruvoyl moiety functions as an electron sink, stabilizes the charge developed during the reaction, and thus assists the decarboxylation. Hydrolysis of this imine causes the release of the amine product and the regeneration of the pyruvoyl enzyme (Figure 1.36).

ThDP is used by various enzymes as a cofactor to perform a wide range of catalytic functions, including the decarboxylation of α-keto acids and transketolation. The thiazolium C-2 atom is deprotonated to form a nucleophilic ylide. The latter attacks the α-carbonyl group of an α-keto acid, forming the first covalent tetrahedral intermediate. Decarboxylation of this intermediate leads to a C-2-α-carbanion which is resonance-stabilized by its enamine form. C-protonation of this enamine intermediate yields a second tetrahedral intermediate. Deprotonation of this intermediate with concomitant cleavage of C-2 of ThDP and C-α of the intermediate finally forms the aldehyde and regenerates the nucleophilic ylide (Figure 1.37).

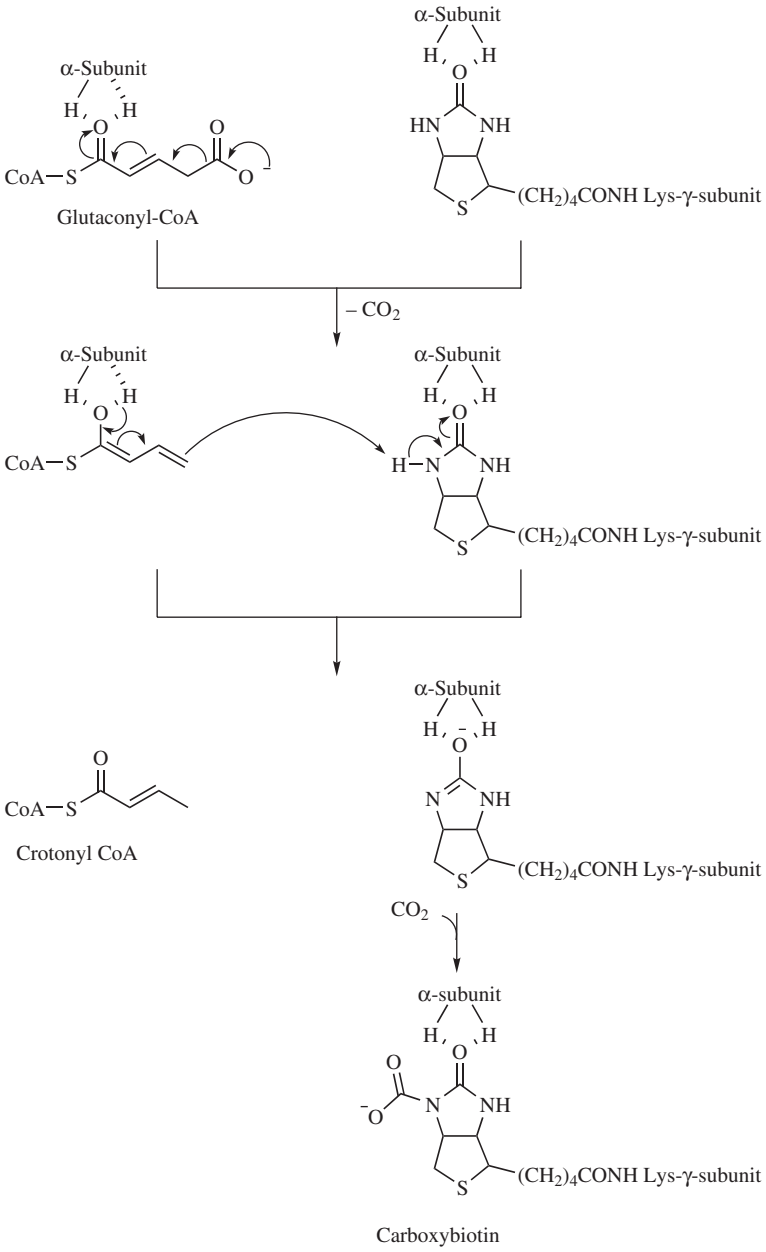


Figure 1.34 Mechanism of decarboxylase–biotin-dependent catalyzed glutaconyl-CoA decarboxylation.

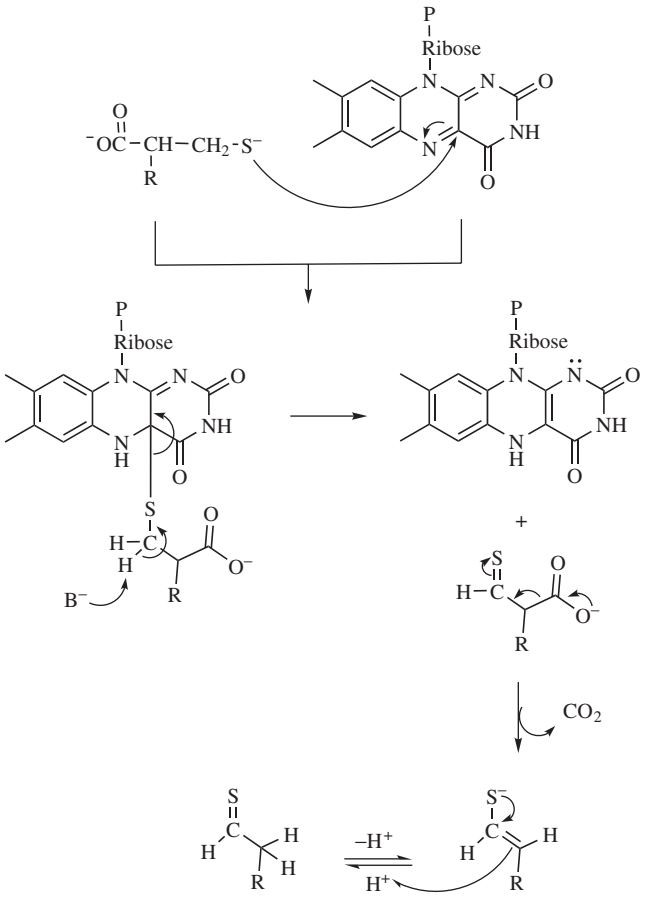


Figure 1.35 Flavin-dependent decarboxylation of cysteine moiety.

1.2.7 Oxidation and Reduction Reactions

Secondary metabolites frequently change their oxidation state during the biosynthesis or degradation of the molecule. The processes may be classified according to the type of enzyme involved and their mechanism of action such as hydrogenation and dehydrogenation reactions.

Hydrogenation/Dehydrogenation Reactions. Hydrogenation (reduction) of an aldehyde, a ketone, or an imine results in a primary alcohol, a secondary alcohol, and an amine, respectively. These reactions are simply nucleophilic additions to a carbonyl or imine, with a hydride ion acting as the nucleophile. On the other hand, the dehydrogenation (oxidation) of a primary alcohol, a secondary alcohol, or an amine results in an aldehyde, a ketone, or an imine, respectively. In dehydrogenation, the hydride is the leaving group. Dehydrogenation and hydrogenation reactions can

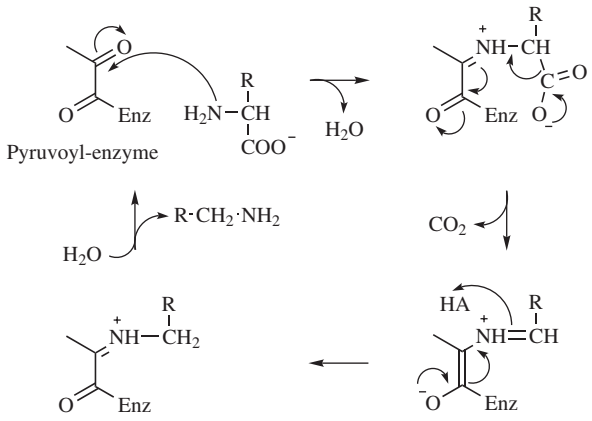


Figure 1.36 Pyruvoyl-dependent decarboxylation of amino acids.

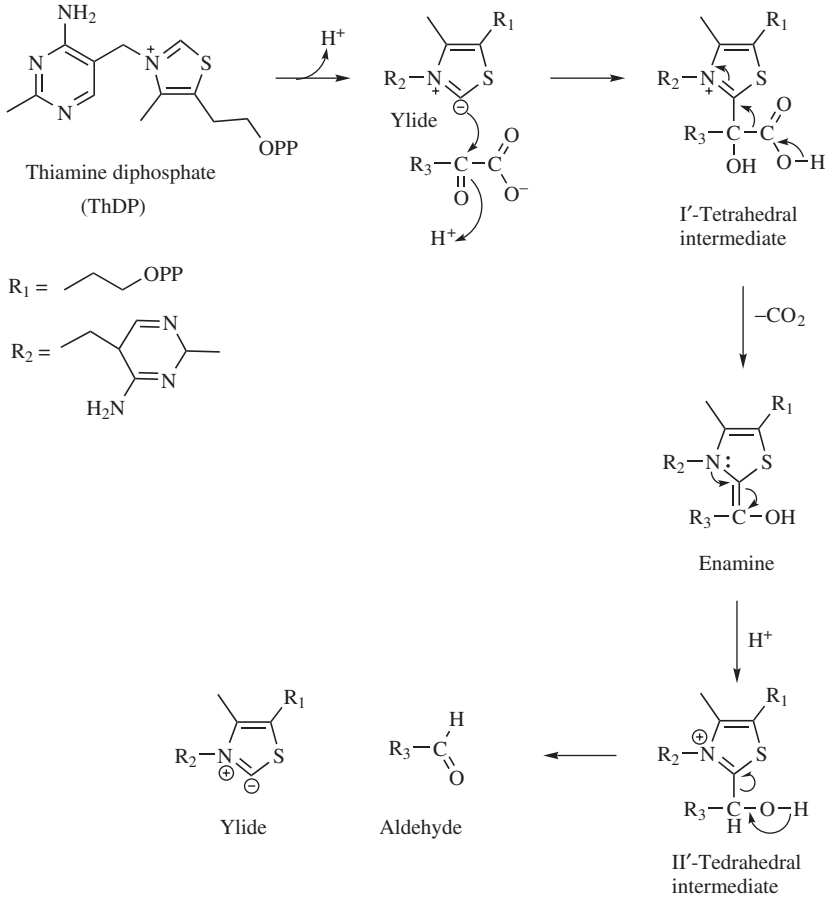


Figure 1.37 ThDP-dependent decarboxylation of α-ketoacids.

be described as hydride transfer reactions. Biochemical redox reactions involving hydride transfer require the participation of a hydride transfer coenzyme such as nicotinamide adenine dinucleotide (NAD) or nicotinamide adenine dinucleotide phosphate (NADP) (Figure 1.38).

The redox reaction occurs specifically at the nicotinamide part of the molecule. The NAD^+ and $NADP^+$ forms act as hydride acceptors (oxidizing agents), whereas the reduced forms NADH and NADPH serve as hydride donors (reducing agents).

In the oxidation process of an alcohol by NAD^+ (or $NADP^+$), the enzymatic base positioned above the carbonyl takes back its proton, and the electrons in the O—H bond shift down and push out the hydride, which is immediately accepted by C-4 of NAD^+ (or $NADP^+$). The products are just what they were started out with, a ketone and NADH (or NADPH) (Figure 1.39). The mechanism of oxidation of amines to imines as well as of aldehyde to carboxylate is similar to the oxidation of alcohol. In the reduction process of a ketone by NADH (or NADPH), both the ketone substrate and cofactor are bound in the enzyme's active site, and C-4 of the nicotinamide ring is positioned very close to the carbonyl carbon of the ketone. As an enzymatic group transfers a proton to the ketone oxygen, the carbonyl carbon becomes more electrophilic and is attacked by a hydride from NADH (or NADPH). The ketone is reduced to an alcohol, and the NADH or NADPH cofactor is oxidized to NAD^+ or

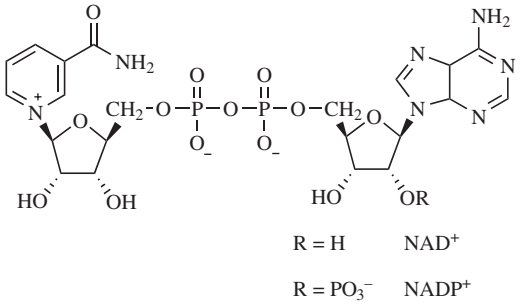


Figure 1.38 Deidrogenases NAD^+ and $NADP^+$.

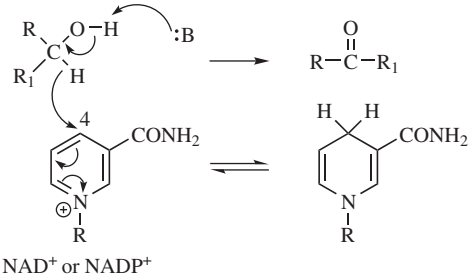


Figure 1.39 Alcohol dehydrogenations.

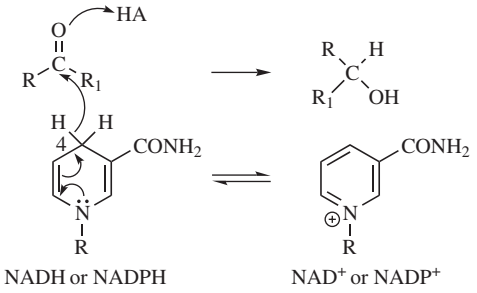


Figure 1.40 Carbonyl hydrogenations.

$NADP^+$ (Figure 1.40). The mechanism of reduction of imines to amines as well as of carboxylate to aldehyde is similar to that of the reduction of ketones.

Instead, biochemical redox reactions involving the oxidation of alkane to alkene require the participation of a coenzyme such as flavin adenine dinucleotide (FAD) or flavin mononucleotide (FMN). The reduced forms $FADH_2$ and $FMNH_2$ act as hydride donors in the alkene hydrogenation reactions (Figure 1.41).

Monooxygenase and Dioxygenase Reactions. In these transformations, which are catalyzed by oxygenase enzymes, one or more oxygen atoms are inserted into a substrate. Mono-oxygenases catalyze the incorporation of one atom of oxygen into the product, while di-oxygenases incorporate both atoms of oxygen into the product(s).

The flavin coenzyme reacts with molecular oxygen to form an intermediate species called *flavin hydroperoxide*. The oxygen of flavin hydroperoxide can be attacked by the π electrons of an alkene group. The subsequent nucleophilic attack of oxygen to the carbocation generates the epoxide ring (Figure 1.42). Epoxides are found as intermediate products in some biosynthetic pathways. The compound

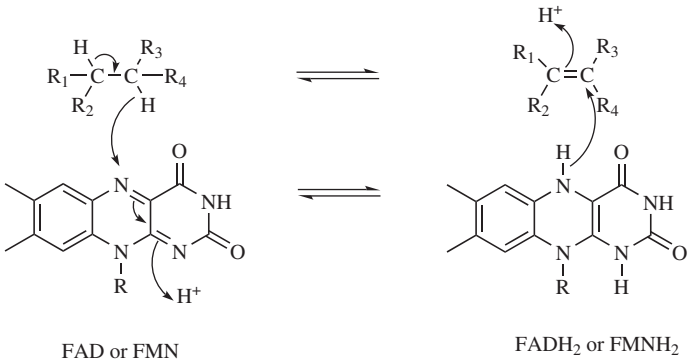


Figure 1.41 Alkane dehydrogenations or alkene hydrogenations.

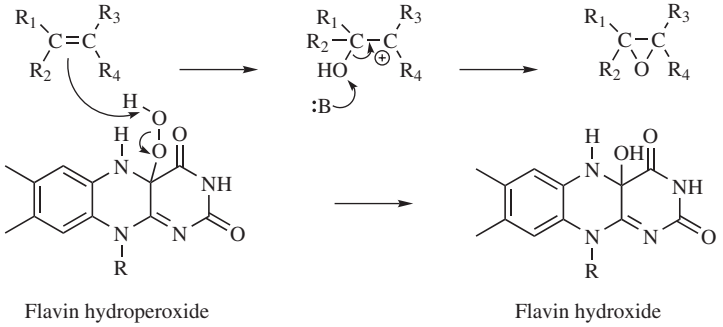


Figure 1.42 Formation of epoxide from an alkene.

(3*S*)-2,3-oxidosqualene, for example, is an important intermediate in the biosynthesis of cholesterol.

Flavin hydroperoxide can also be attacked by the π electrons of an aromatic group. For example, in the oxidation of tryptophan, the intermediate (kynurenine) is hydroxylated via an electrophilic aromatic substitution with the reactive oxygen of flavin hydroperoxide (Figure 1.43).

Degradation of aromatic compounds is one of the most important functions of dioxygenases. The substrates of ring-cleavage dioxygenases can be classified into two groups according to the mode of scission of the aromatic ring in intradiol-

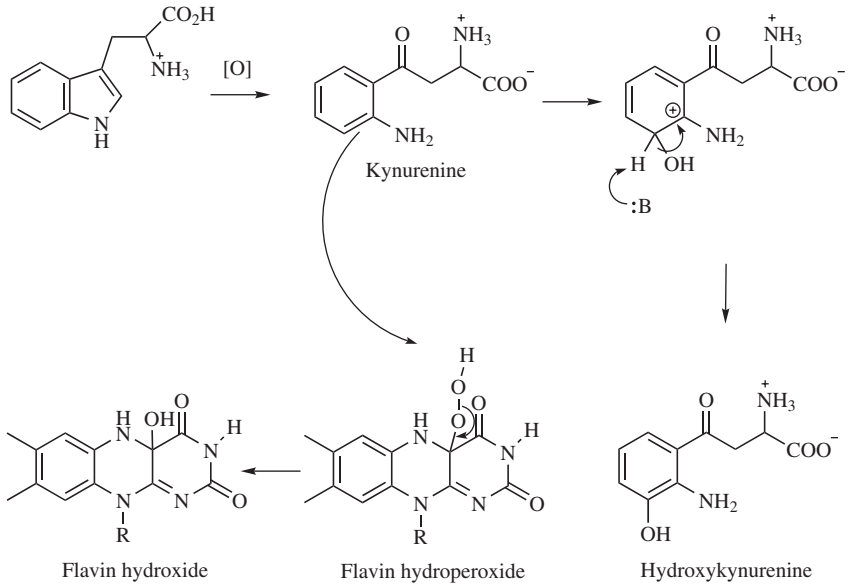


Figure 1.43 Oxidation of kyrunenine in tryptophan biosynthesis.

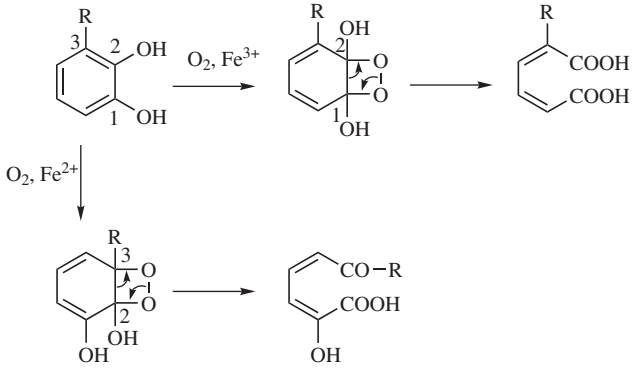


Figure 1.44 Reaction catalyzed by intradiol and estradiol dioxygenases.

and extradiol-cleaving enzymes. Intradiol dioxygenases, typified by catechol 1,2-dioxygenase, cleave the carbon–carbon bond between the phenolic hydroxyl groups and require Fe(III) as a cofactor, whereas extradiol dioxygenases, typified by catechol 2,3-dioxygenase, cleave the carbon–carbon bond adjacent to the phenolic hydroxyl groups and require Fe(II) as a cofactor (Figure 1.44) [35].

Another example of dioxygenase reaction is the degradation of tryptophan to *N*-formylkynurenine by indoleamine 2,3-dioxygenase (IDO) and tryptophan 2,3-dioxygenase (TDO). A ternary complex is initially formed by dioxygen binding to the ferrous protein. The formation of the hydroperoxide intermediate is catalyzed by the loss of the indole proton. The subsequent rearrangement of the hydroperoxide intermediate to form the product could occur via either a Criegee or a dioxetane intermediate. Thermodynamic considerations and chemiluminescence support the Criegee rearrangement (Figure 1.45) [36].

Baeyer–Villiger Reactions. Baeyer–Villiger oxidation is the oxidative cleavage of a carbon–carbon bond adjacent to a carbonyl, and converts ketones to esters and cyclic ketones to lactones (Figure 1.46). The enzymes that catalyze these reactions are Baeyer–Villiger monooxygenases (BVMOs), which are NADPH-dependent flavoproteins.

During the enzymatic oxidation, one atom of molecular oxygen is incorporated on the carbon adjacent to the non-activated ketone, whereas the other oxygen atom ends up in a water molecule with the hydrogen atoms originating from the cofactor NADPH. The protein-bound FAD is reduced by NADPH, generating the reduced enzyme–NADP complex. This binary complex reacts with oxygen to form a flavin peroxide species which undergoes a nucleophilic attack on the carbonyl group of the ketone substrate. The Criegee intermediate thus formed rearranges to the ester product with the concomitant formation of a flavin hydroxide. Finally, water is eliminated from the latter species to re-form oxidized FAD, and the release of NADP completes the catalytic cycle (Figure 1.47).

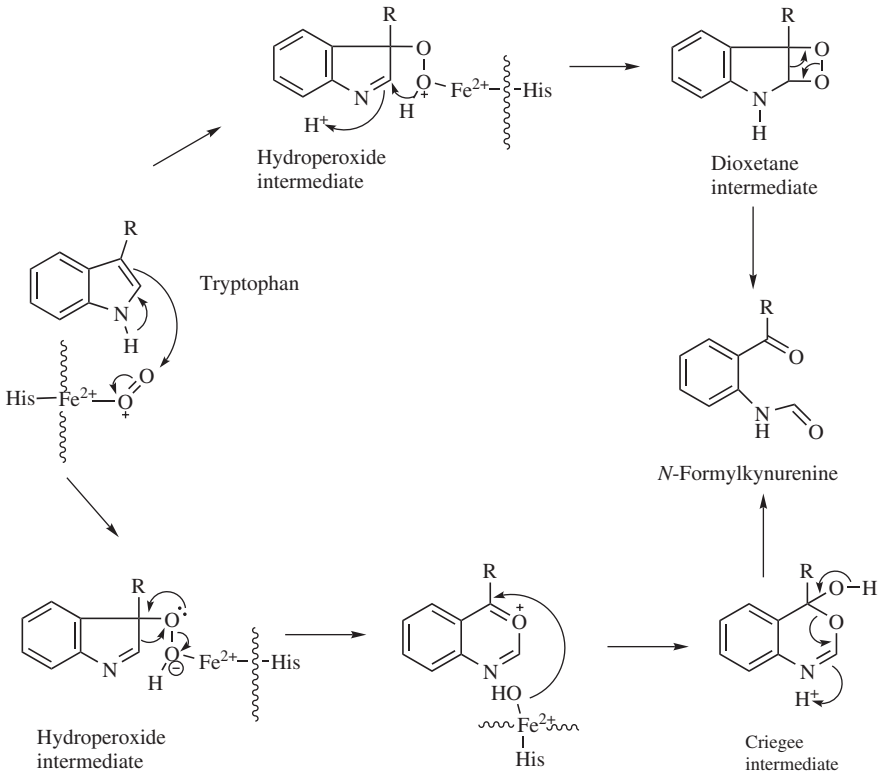


Figure 1.45 Proposed catalytic tryptophan degradation.

Figure 1.46 Enzymatic Baeyer–Villiger oxidations.

The earliest report of a "biological" Baeyer–Villiger reaction is probably the conversion of progesterone to testololactone by several microorganisms (Figure 1.48).

Another important example is provided by the biosynthesis of gilvocarcins (natural anticancer antibiotics). The proposed biosynthesis of dihydroxyphenylglyoxylate, the building block of gilvocarcins, goes through a Baeyer–Villiger oxidation. The reaction is sequential and requires 2 equiv of $FADH_2$ since the enzymes catalyze both the initial 5-hydroxylation and the following Baeyer–Villiger oxidation (Figure 1.49) [37].

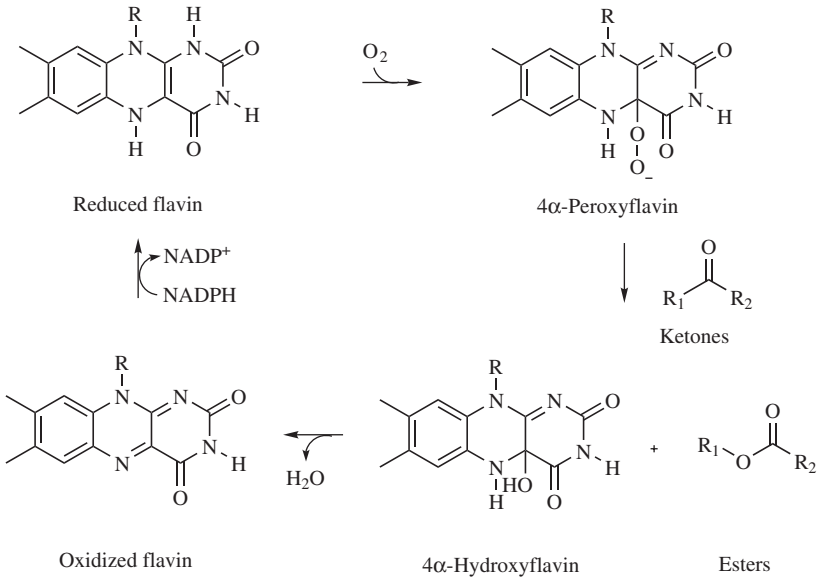


Figure 1.47 Flavin-dependent Baeyer–Villiger monooxygenase-mediated oxidation reactions.

Further examples of BVMO reactions are reported in the biosynthesis of urdamycin (the antitumor antibiotic produced by the soil bacteria *Streptomyces fradiae* TU 2717) [38], jadomycin (a glycoside antibiotic produced by the soil bacterium *Streptomyces venezuelae* ISP5230) [39], and 5-alkenyl-3,3(2*H*)-furanones (isolated from *Streptomyces aculeolatus* NRRL 18422 and *Streptomyces* sp. Eco86) [40].

Oxidative Deamination Reactions. The enzymes amine oxidases (AOs) catalyze the oxidative deamination of a wide range of biogenic amines. There are two classes of AOs: copper AOs and flavin-containing AOs. Copper-containing AOs catalyze the oxidation of primary amines to aldehydes, with the subsequent release of ammonia and hydrogen peroxide, which requires one copper ion per subunit and topaquinone (TPQ) (2,4,5-trihydroxyphenylalanine) as cofactor (Figure 1.50).

The carbonyl of TPQ reacts with the primary amine, forming the SB intermediate. This SB is then tautomerized to generate a product Schiff base (PSBa) which is in resonance equilibrium with form b (PSBb). Hydrolyses of the latter generate the aldehyde and afford a reduced aminated topaquinone (TPQamr). This mechanism is known as the *reductive half-reaction*. In the oxidative half-reaction, the TPQamr is oxidized by O_2 to form quinonimine, which is hydrolyzed to give NH_3 and regenerate the TPQ cofactor (Figure 1.51) [41].

Monoamine oxidases (MAOs) are examples of flavin-containing AOs that catalyze the oxidation of amines to the corresponding aldehyde and NH_3 using O_2 as

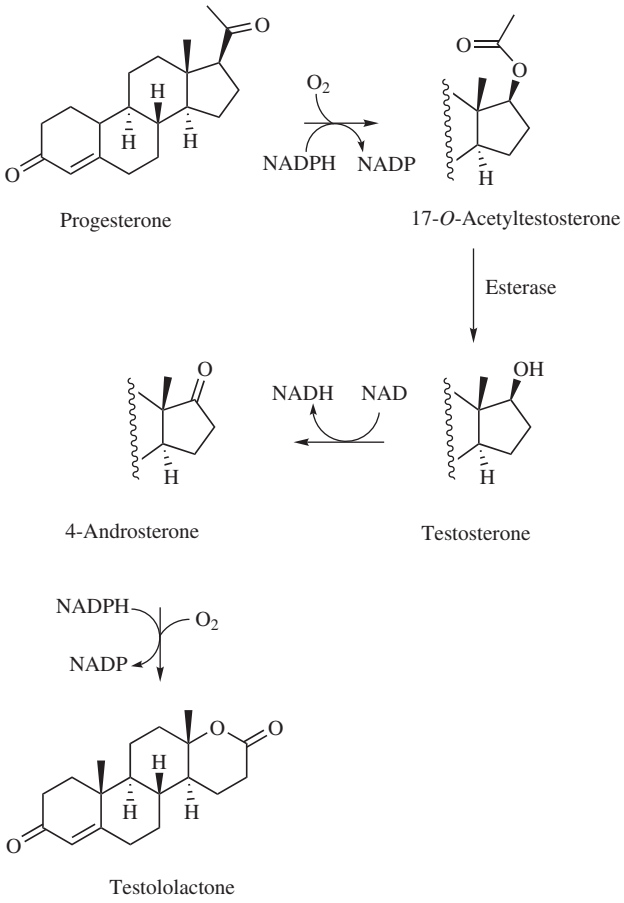


Figure 1.48 Baeyer–Villiger oxidation of steroids.

an electron acceptor (Figure 1.52) [42]. The mechanism for MAO catalysis may be applicable to the reaction mechanisms of other amine-oxidizing flavoenzymes.

1.2.8 Dehalogenation/Halogenation Reactions

Dehalogenases catalyze the substitution of a halogen by water in a nucleophilic replacement reaction which generates an alcohol. The more studied hydrolytic halogenase is the haloalkane dehalogenase (Figure 1.53). In the reaction mechanism, the enzyme contains a catalytic triad consisting of one histidine and two aspartate residues involved in the nucleophilic substitution of the halogen atom. Moreover, two tryptophan residues facilitate the removal of the halide ion (Figure 1.54) [43].

Another example is furnished by the dehalogenation of dichloromethane by glutathione-*S*-transferase (GSH), which catalyzes the formation of an unstable

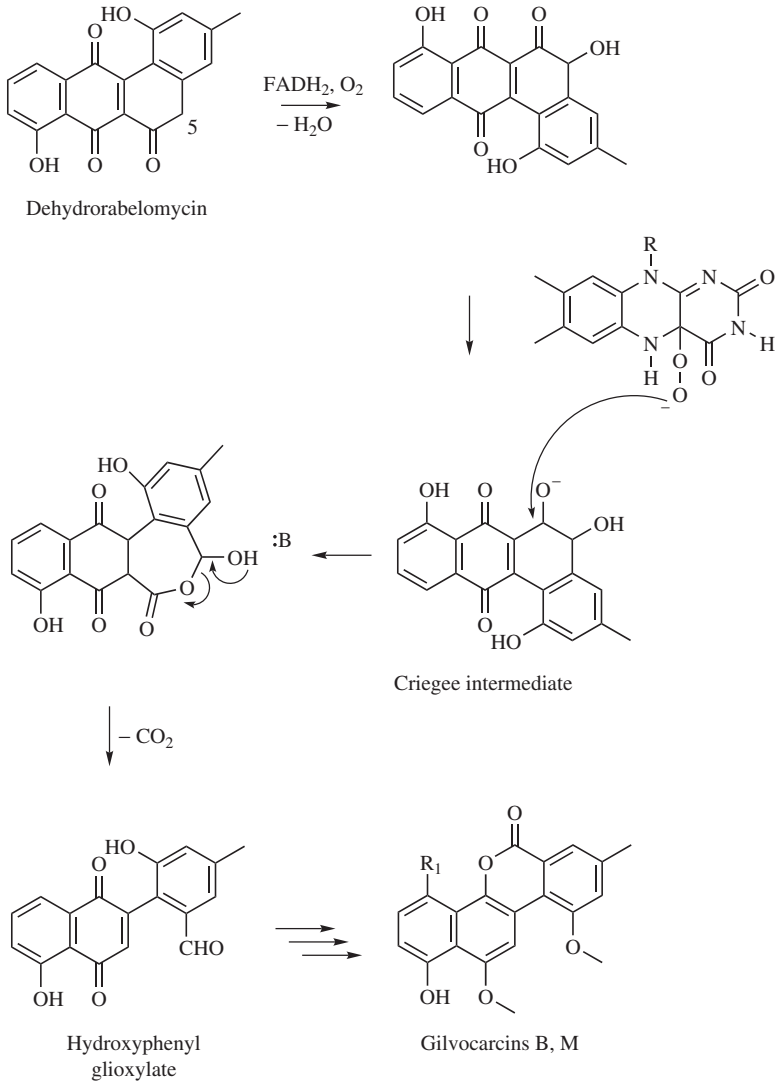


Figure 1.49 Proposed biosynthesis of dihydroxyphenylglyoxylate, building block of gilvocarcins B and M, via a Baeyer–Villiger oxidation.

Figure 1.50 Deamination reactions.

S-chloromethyl GSH intermediate. The latter is hydrolyzed to GSH chloride, and formaldehyde (Figure 1.55) [44, 45]. Haloalcohol dealogenases (halohydrin dehalogenases) catalyze the intramolecular nucleophilic displacement of a halogen by a vicinal hydroxyl group to form epoxides [46]. These enzyme show sequence

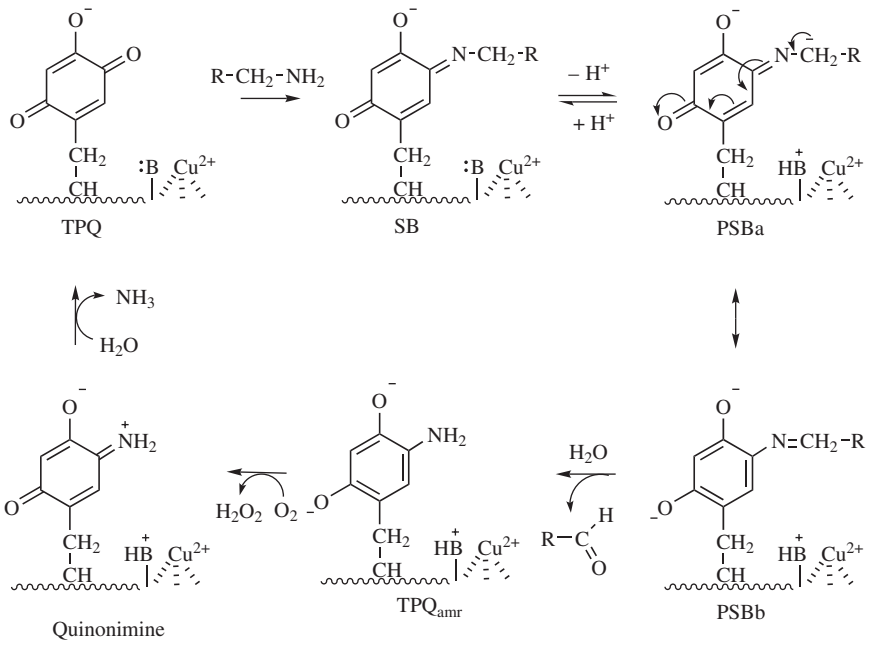


Figure 1.51 Mechanism of deamination by copper amine oxidases.

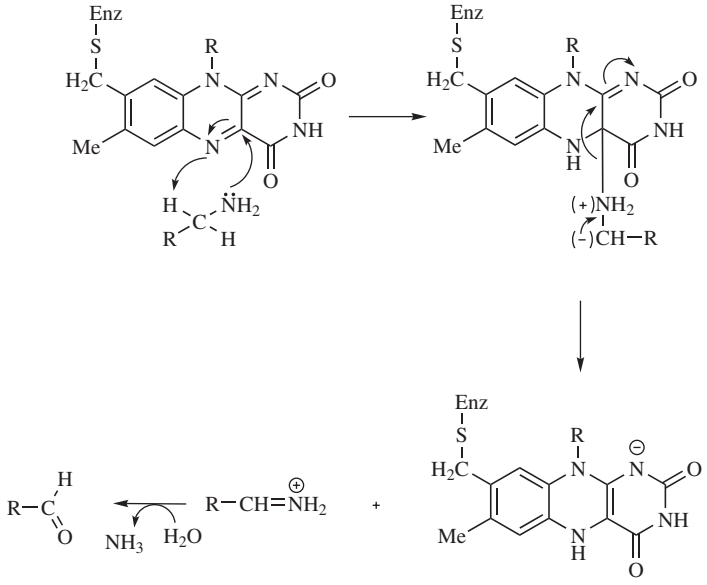


Figure 1.52 Mechanism of deamination by MAO.

Figure 1.53 Hydrolytic dehalogenation.

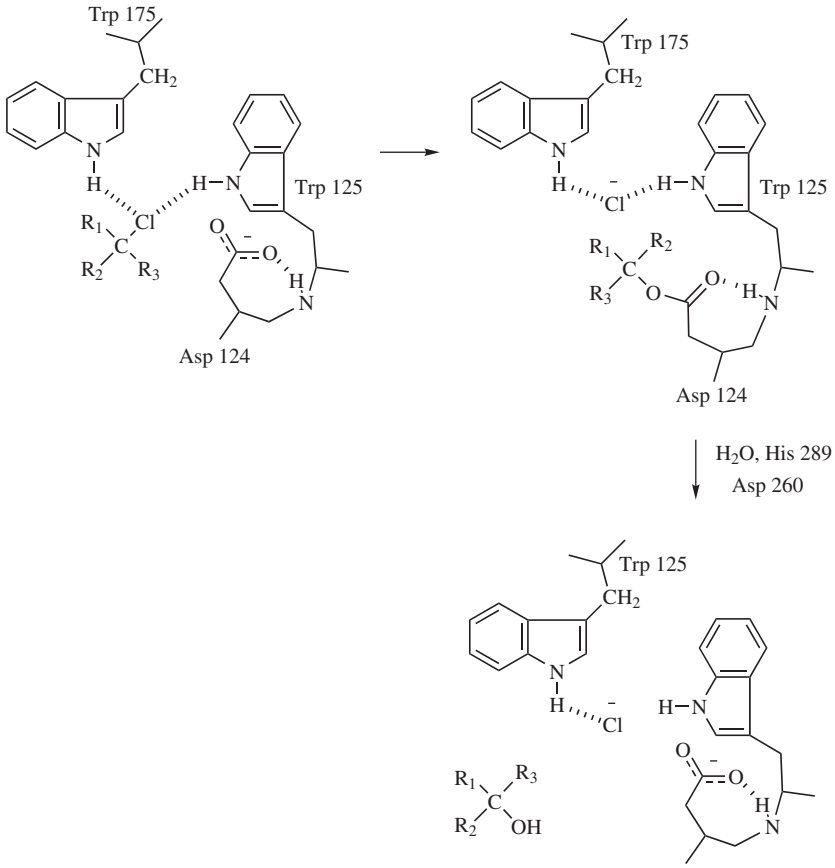


Figure 1.54 Mechanism of haloalkane dehalogenase.

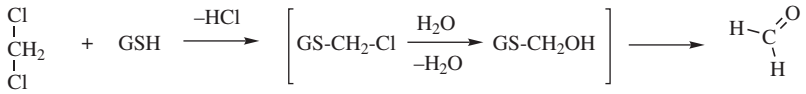


Figure 1.55 Thiolytic dehalogenation of dichloromethane by GSH.

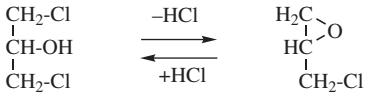


Figure 1.56 Dehalogenations/halogenations by haloalcohol dehalogenases.

homology with the family of dehydrogenases/reductases (they do not require $NADP^+$ as a coenzyme). They can also catalyze the reverse reaction, namely the halogenation of epoxide to haloalcohols (Figure 1.56) [47].

Biological halogenation occurs on a diverse array of organic scaffolds. Within these scaffolds, halogen atoms are incorporated on aliphatic carbons, olefinic centers, as well as aromatic and heterocyclic rings. The first isolated halogenating enzyme was cloroperoxidase, which requires hydrogen peroxide and halide ions (chloride, bromide, iodine). Elucidation of 3D structure and biomimetic studies revealed that heme-type haloperoxidases produce free hypohalous acids (HOX; X = Cl, Br, or I) as the halogenating agent (Figure 1.57) [48].

$FADH_2$-dependent halogenases play a significant role in the halogenation of aromatic moieties. They require reduced $FADH_2$, a halide ion (Cl^- or Br^-), and O_2 as co-substrates for halogenation reaction. $FADH_2$ reacts with O_2 to form the 4α-hydroperoxyflavin (FAD-4α-OOH) intermediate (Figure 1.58a). Two reaction mechanisms have been proposed for the flavin-dependent halogenases. The first involves the nucleophilic mechanism, with the initial formation of an epoxide from the reaction of the aromatic substrate with the FAD-4α-OOH intermediate. A subsequent nucleophilic attack of a halogen atom leads to the formation of a

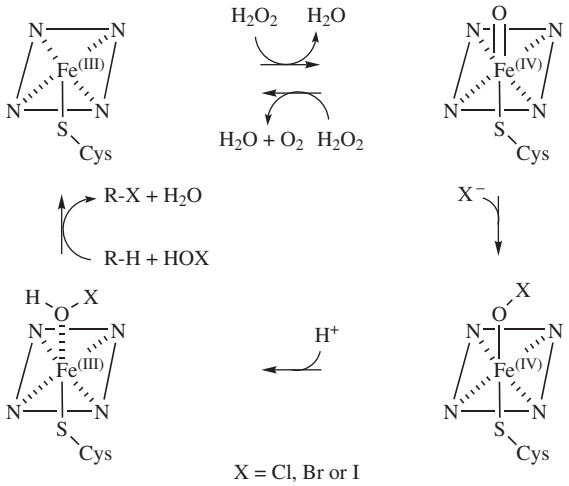


Figure 1.57 Reaction mechanisms of heme-type haloperoxidases showing the formation hypohalous acids (HOX; X = Cl, Br, or I).

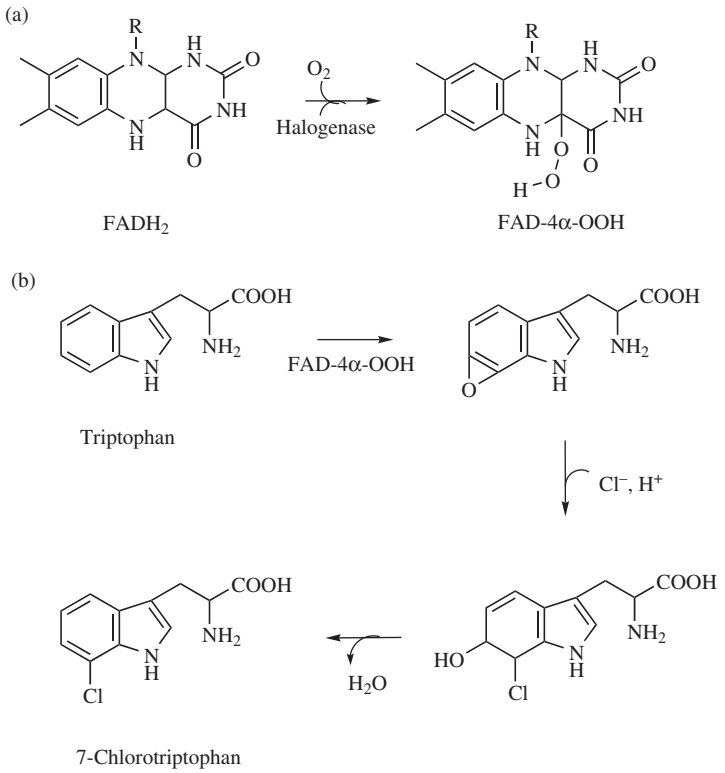


Figure 1.58 Halogenation reactions catalyzed by flavin-dependent halogenase. (a) Formation of FAD-4α-OOH intermediate and (b) nucleophilic mechanism of halogenation.

halohydrin which dehydrates to give the final halogenated product (Figure 1.58b). In the second proposed mechanism, the FAD-4α-OOH intermediate reacts with a halide ion to form FAD-4α-OH and the halogenating species hypochlorous acid (HOCl) (Figure 1.59a) [49, 50]. In the tryptophan chlorination catalyzed by tryptophan 7-halogenase, the Lys-79 residue provides a hydrogen bond to the HOCl; in this manner, chlorine is activated by increased electrophilicity. Electrophilic addition of chlorine to tryptophan (stabilized by a glutamate residue) and the subsequent deprotonation by Glu-346 of the intermediate give 7-chlorotryptophan (Figure 1.59b).

Tryptophan chlorination can be also catalyzed by the halogenating enzyme RebH. In this case, HOCl reacts with the active site Lys79 residue of RebH to form a lysine chloramine Lys-ϵNH-Cl. This intermediate delivers a Cl^+ equivalent for the electrophilic aromatic substitution of the tryptophan indole ring at C-7 position (Figure 1.59c) [50]. The nonheme Fe(II) O_2- and α-ketoglutarate (αKG)-dependent enzymes catalyze the halogenation of aliphatic C atoms. Fe(II) is bound by two histidine residues. A halide ligand (Cl^- or Br^-) together with αKG and H_2O coordinates Fe(II). An O_2 attack results in the decarboxylation of αKG and the generation of the highly reactive species Fe(IV)=O. The latter extracts a hydrogen

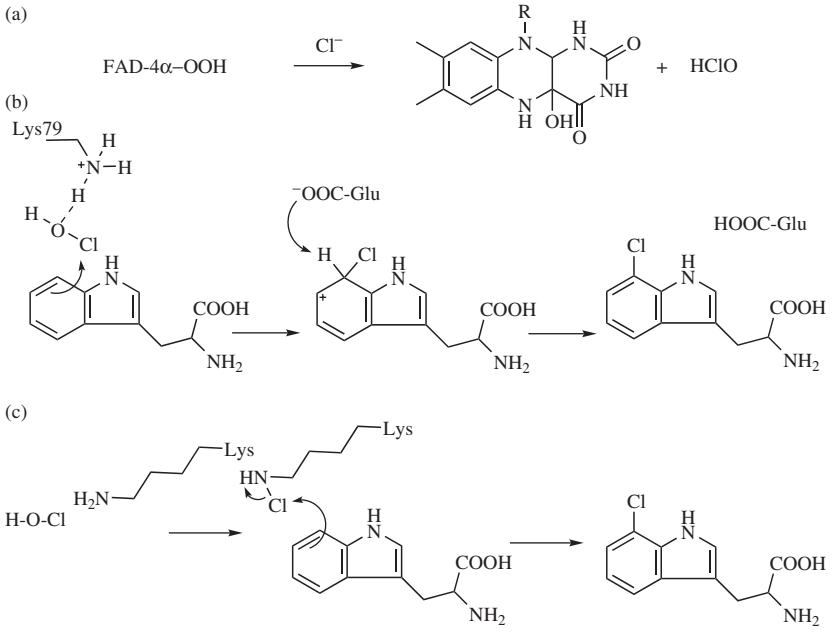


Figure 1.59 Formation of hypochlorous acid from FAD-4α-OOH (a) mechanism of tryptophan chlorination (b) catalyzed by tryptophan 7-halogenase and (c) catalyzed by halogenating enzyme RebH.

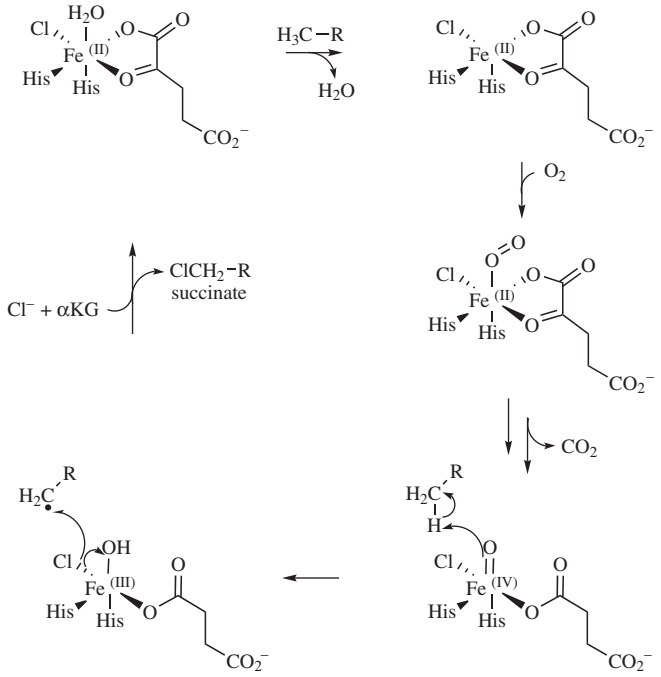


Figure 1.60 Proposed mechanism for nonheme Fe(II), O_2, and α-ketoglutarate-dependent halogenation.

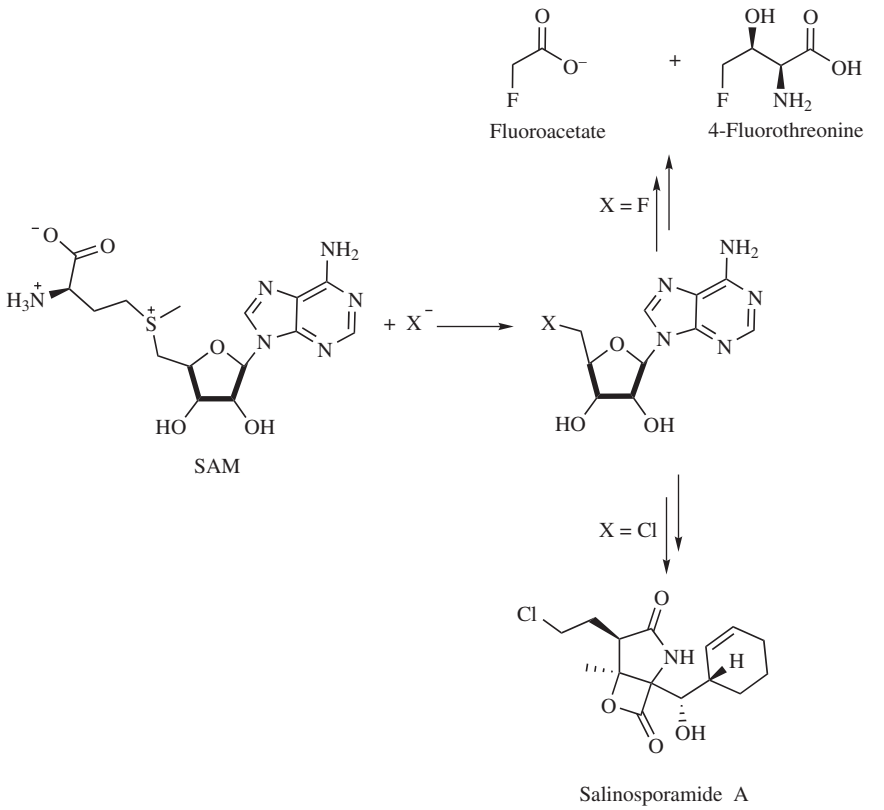


Figure 1.61 Halogenating enzymes utilizing SAM.

radical from the terminal methyl group, which in turn extracts the radical halide from the coordination sphere (Figure 1.60) [51]. Further examples of this type of halogenations are reported in the biosynthesis of the barbamides dysidenin and dysideathiazole and jamaicamides [52, 53].

The enzyme 5′-fluoro-5′-deoxyadenosine synthetase (5′-FDAS), utilizing SAM, catalyzes the formation of carbon–fluoride bonds. In fact, in presence of F^-, an S_N2 substitution of methionine at SAM generates the intermediate 5′-fluoro-5′-deoxyadenosine, which is subsequently converted to fluoroacetate and 4-fluorothreonine, whereas in the biosynthesis of salinosporamide A the enzyme achieves the chlorination step through the conversion to 5′-chloro-5′-deoxyadenosine (Section 4.2.2) (Figure 1.61) [51].

1.2.9 Glycosylation Reactions

Glycosylation is a reaction in which a sugar unit is covalently attached to the aglycone through O-, N-, S-, or C-glycosidic linkages. *O*-glycosides are the most common ones.

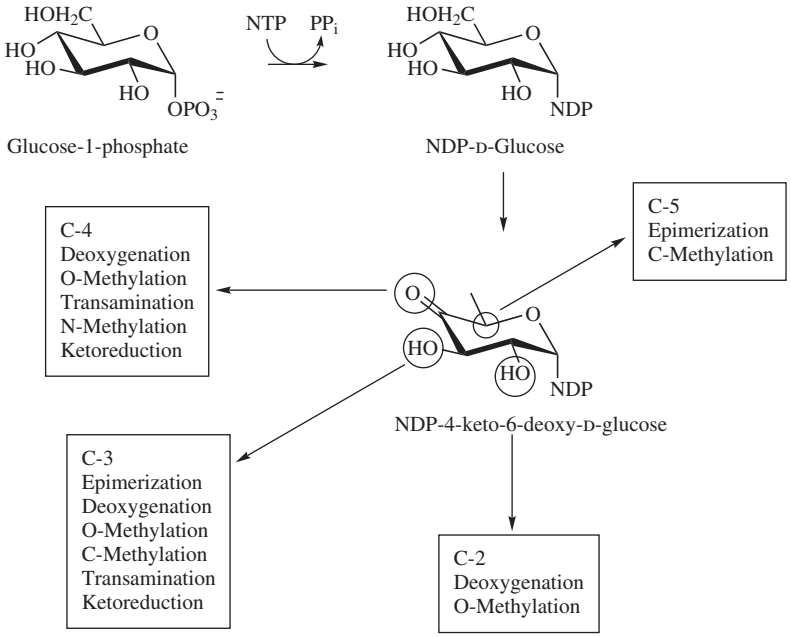


Figure 1.62 Biosynthesis of NDP sugars from sugar 1-phosphate and reaction originating from NDP-4-keto-6-deoxy-D-sugars.

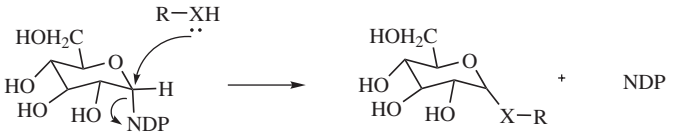
Glycosylation can occur with one or more sugar units with variable chain lengths and at different positions. Once the sugar is incorporated by the glycosyltransferase, it can be further modified by enzymatic reactions such as deoxygenation, epimerization, oxidation/reduction, transamination, methylation, and acylation. The combinations of all these enzymatic reactions generate a variety of structurally diverse glycosylated compounds.

Nucleotide 5′diphosphosugars (NDP sugars) represent the most common form of sugar donor employed by glycosyltransferases. The formation of an NDP sugar catalyzed by a dNDP-sugar-1-phosphate (deoxynucleoside-diphosphate) nucleotidylyltransferase derives from the action of sugar-1-phosphate with nucleoside triphosphate (NTP) via expulsion of PP.

The dNDP-D-sugar-4,6-dehydratase catalyzes the formation of the common intermediate dNDP-4-keto-6-deoxy-D-sugar. Biosynthesis of most glycosylated natural compounds derives from the dNDP-activated sugar (mainly D-glucose) through 4-keto-6-deoxy intermediates (Figure 1.62) [54, 55].

Glycosylation is produced by an S_N2 nucleophilic displacement reaction where NDP is the leaving group (Figure 1.63).

Some examples of glycosylation of natural products are doxorubicin, erythromycin, staurosporine, urdamycin A, saccharomicin, and landomycin.



X = O, NH, S, C

Figure 1.63 Glycosylation reactions.

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