Role of Cytochrome P_{450} monooxygenase in Oxidative Biotransformation:

Oxidative biotransformation processes are the most common and important in drug metabolism. The enzyme systems carrying out this biotransformation are referred to as *mixed-function oxidases* or *monooxygenases*.

Nomenclature of these enzymes is as the following:

There are four components to the name.

- CYP refers to the cytochrome system.
- This is followed by the number that specifies the cytochrome family (CYP1, CYP2, CYP3, etc.).
- Next is a capital letter that represents the subfamily (CYP1A, CYP1B, CYP2A, CYP2B, CYP3A, CYP3B, etc.).
- Finally, the cytochrome name ends with another number that specifies the specific enzyme responsible for a particular reaction (CYP1A2, CYP2C9, CYP2C19, CYP3A4, etc.).

$$\underset{\text{Xenobiotic}}{RH} + ADPH + O_2 \longrightarrow H^+ + \underset{\text{oxidized metabolite}}{ROH} + NADP + H_2O$$

The reaction requires both molecular oxygen and the reducing agent NADPH (reduced form of nicotinamide adenosine dinucleotide phosphate). During this oxidative process, one atom of molecular oxygen (O2) is introduced into the substrate R-H to form R-OH and the other oxygen atom is incorporated into water. The mixed-function oxidase system is actually made up of several components, the most important being the superfamily of CYP oxidase enzymes which are responsible for transferring an *oxygen atom* to the substrate RH.

Other important components of this system include the NADPH-dependent CYP reductase and the NADH-linked cytochrome b5. These two components, along with the cofactors NADPH and NADH, supply the reducing equivalents (electrons) needed in the overall metabolic oxidation of foreign compounds.

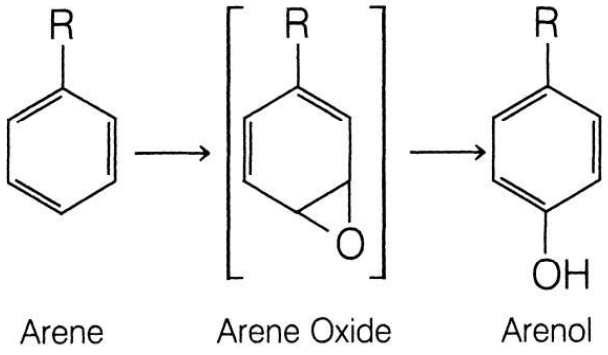
The CYP enzymes are heme proteins. The heme portion is an iron-containing porphyrin called *protoporphyrin IX*, and the protein is called the *apoprotein*. CYP is found in high concentrations in the liver, the major organ involved in the metabolism of xenobiotics. The presence of this enzyme in many other tissues (e.g., lung, kidney, intestine, skin, placenta, adrenal cortex) shows that these tissues have drug-oxidizing capability too. The name *cytochrome P450* is derived from the fact that the reduced (Fe^{+2}) form of this enzyme binds with carbon monoxide to form a complex that has a distinguishing spectroscopic absorption maximum at 450 nm.

One important feature of the hepatic CYP mixed function oxidase system is its ability to metabolize an almost unlimited number of diverse substrates by various oxidative transformations. This versatility is believed to be a result of the substrate nonspecificity of CYP as well as the presence of multiple forms of the enzyme. Some of these P450 enzymes are selectively inducible by various chemicals.

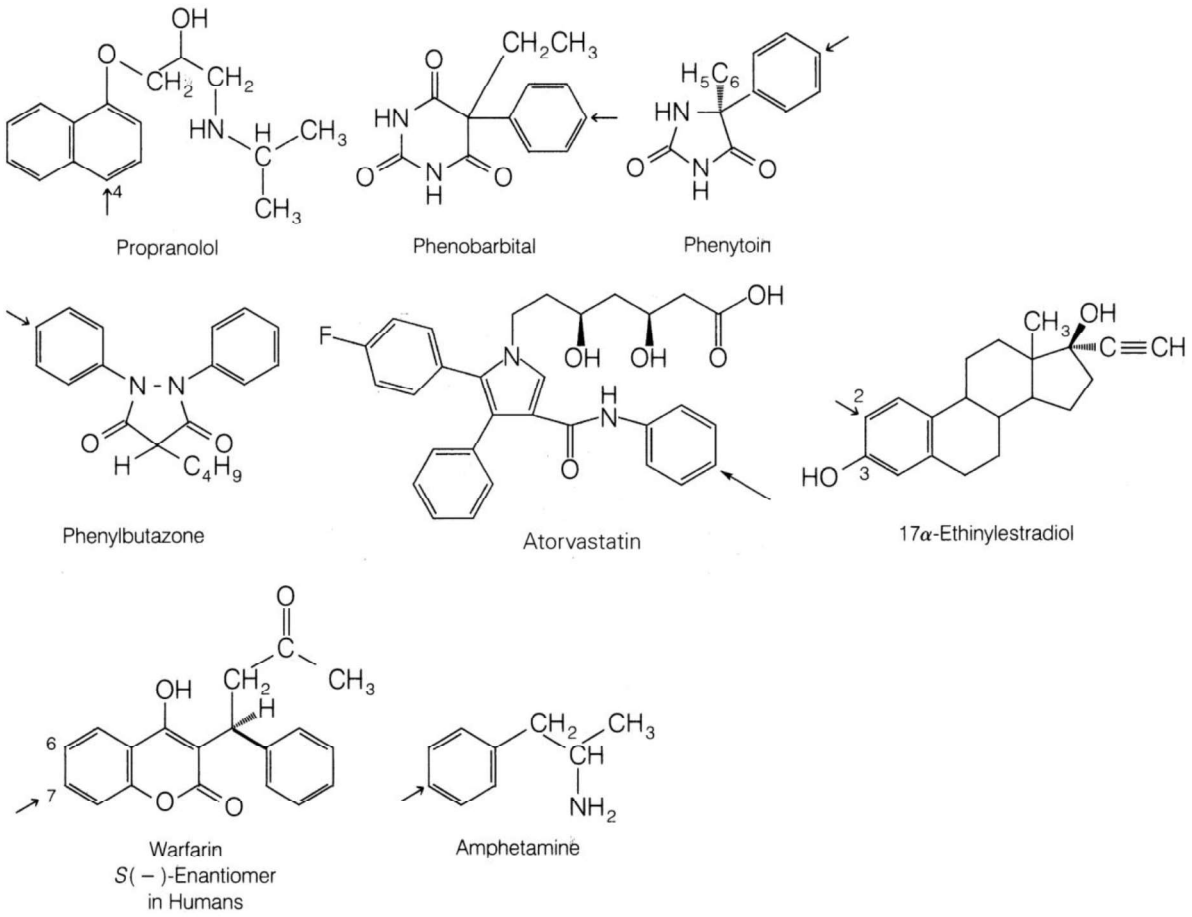
Phase I Oxidation reaction

1. Oxidation of Aromatic Moieties

Aromatic hydroxylation refers to the mixed-function oxidation of aromatic compounds (arenes) to their corresponding phenolic metabolites (arenols). Almost all aromatic hydroxylation reactions are believed to proceed initially through an epoxide intermediate called an "arene oxide," which rearranges rapidly and spontaneously to the arenol product in most instances.



Most foreign compounds containing aromatic moieties are susceptible to aromatic oxidation. In humans, aromatic hydroxylation is a major route of metabolism for many drugs containing phenyl groups. Important therapeutic agents such as propranolol, phenobarbital, phenytoin and atorvastatin, undergo extensive aromatic oxidation. In most of these drugs hydroxylation occurs at the para position.



Examples of drugs and xenobiotics that undergo aromatic hydroxylation in humans. *Arrow* indicates site of aromatic hydroxylation.

Factors affecting aromatic oxidation:

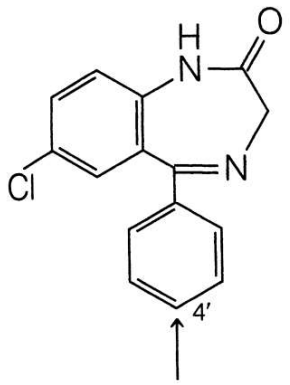
The substituents attached to the aromatic ring may influence the ease of hydroxylation.

Types of substitutions are classified to

- Activated group: like hydroxyl, amine, alkyl, etc. (electron-donating group). Cause ring activation, in this case oxidation is characterized by rapid metabolism and position of OH group at para position.
- Deactivation group: like halogens, NO_2, ammonium ion, COOH, SO_2NHR, etc. (electron-withdrawing group) are generally slow or resistant to hydroxylation.

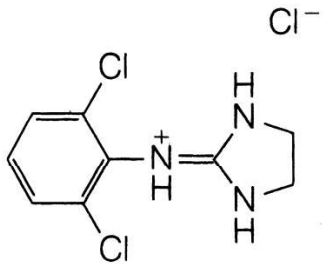
Examples:

e.g.1: Diazepam, compounds with two aromatic rings, hydroxylation occurs preferentially in the more electron-rich ring.



Diazepam

e.g.2: The deactivating groups (Cl, $-N^+H=C$) present in the antihypertensive clonidine may explain why this drug undergoes little aromatic hydroxylation in humans.



Clonidine Hydrochloride

Arene oxide intermediates are formed when a double bond in aromatic moieties is epoxidized. Arene oxides are of significant toxicologic concern because these intermediates are electrophilic and chemically reactive (because of the strained three-membered epoxide ring).

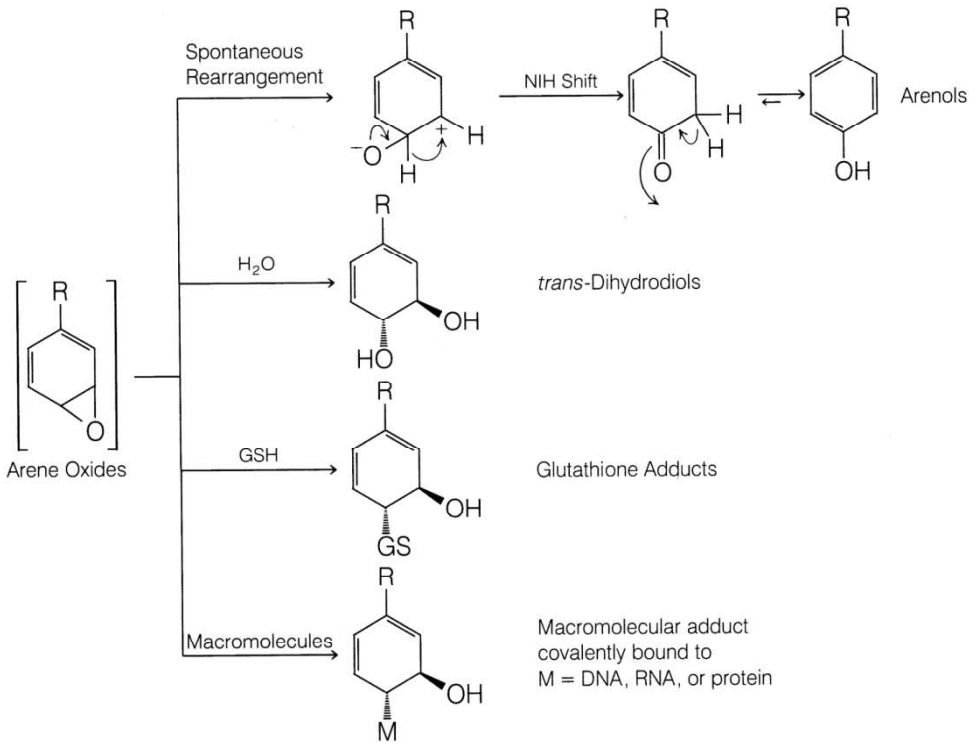
Arene oxides are mainly detoxified by the following possible reaction pathways:

a- The most important detoxification reaction for arene oxides is the spontaneous rearrangement to corresponding arenols. Often, this rearrangement is accompanied by a novel intramolecular hydride migration called the “NIH shift.”

b- Enzymatic hydration to trans-dihydrodiols. (i.e., nucleophilic attack of water on the epoxide). Transdiol may undergo further oxidation to give catechol derivative. This reaction is catalyzed by microsomal enzymes called epoxide hydrases.

c- Enzymatic conjugation with (GSH) in the presence of glutathione S-trnsferase enzyme to give glutathione derivatives, which undergo further metabolism to give mercapturic derivative.

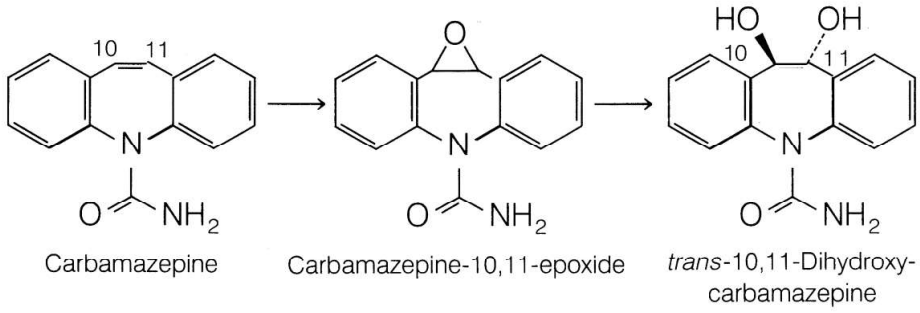
If not effectively detoxified by the first three pathways, arene oxides will bind covalently with nucleophilic groups present on proteins, (DNA), and (RNA), thereby leading to serious cellular damage. This, in part, helps explain why benzene can be so toxic to mammalian systems.



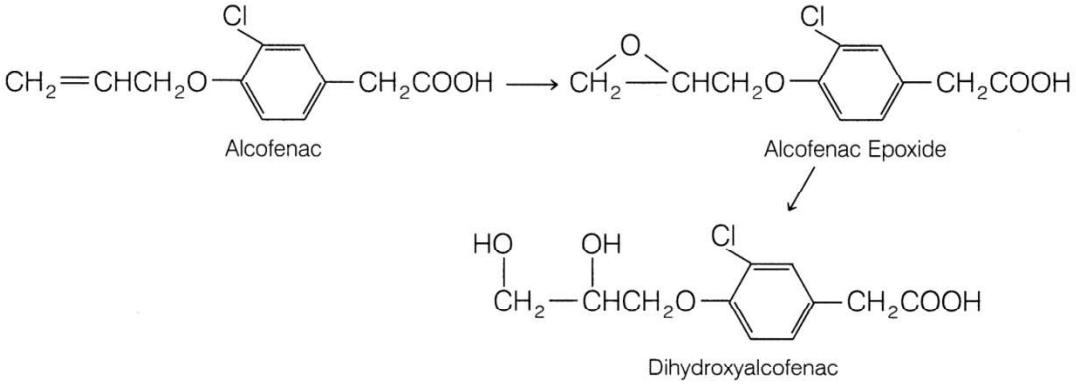
Possible reaction pathways for arene oxides

2. Oxidation of Olefin

The metabolic oxidation of olefinic carbon-carbon double bonds leads to the corresponding epoxide (or oxirane). Epoxides are susceptible to enzymatic hydration by epoxide hydrase to form *trans*-dihydrodiols. In addition, several epoxides undergo GSH conjugation. **E.g.** The anticonvulsant drug Carbamazepine (Tegretol) and Alcofenac.

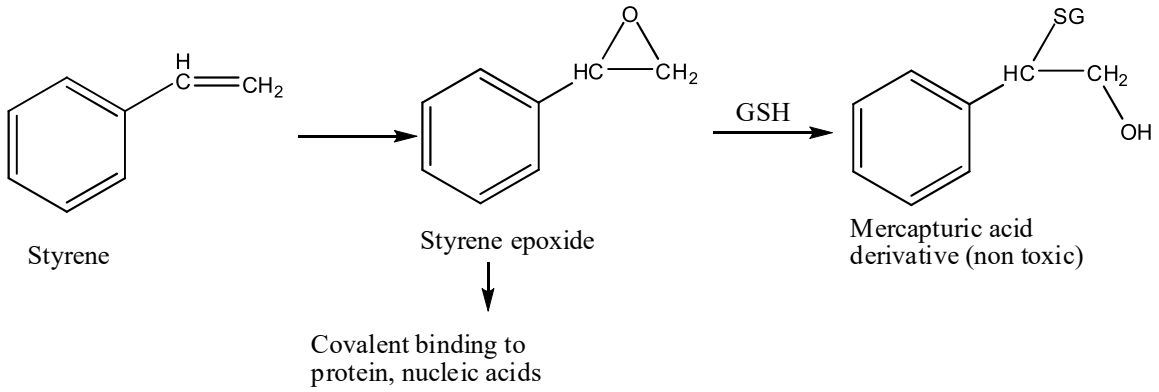


Carbamazepine → Carbamazepine-10,11-epoxide → *trans*-10,11-Dihydroxy-carbamazepine



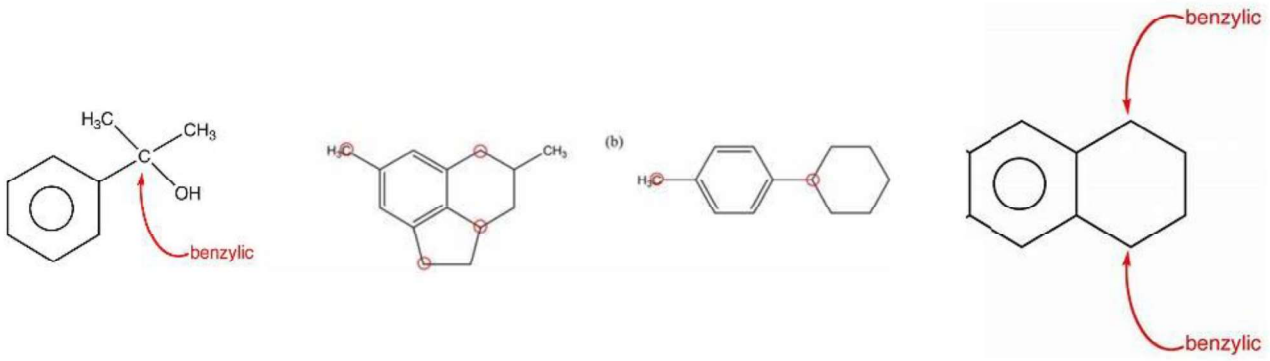
Alcofenac → Alcofenac Epoxide → Dihydroxyalcofenac

Other olefinic compounds, such as vinyl chloride, stilbene undergoes metabolic epoxidation. The corresponding epoxide metabolites may be the reactive species responsible for the cellular toxicity seen with these compounds.



Some of olefin-containing compounds causes the destruction of CYP. Such compounds are secobarbital and the volatile anesthetic agent fluroxene. It is believed that the olefinic moiety present in these compounds is activated metabolically by CYP to form a very reactive intermediate that covalently binds to the heme portion of CYP. Long-term administration of the above-mentioned agent is expected to lead to inhibition of oxidative drug metabolism, potential drug interactions, and prolonged pharmacological effects.

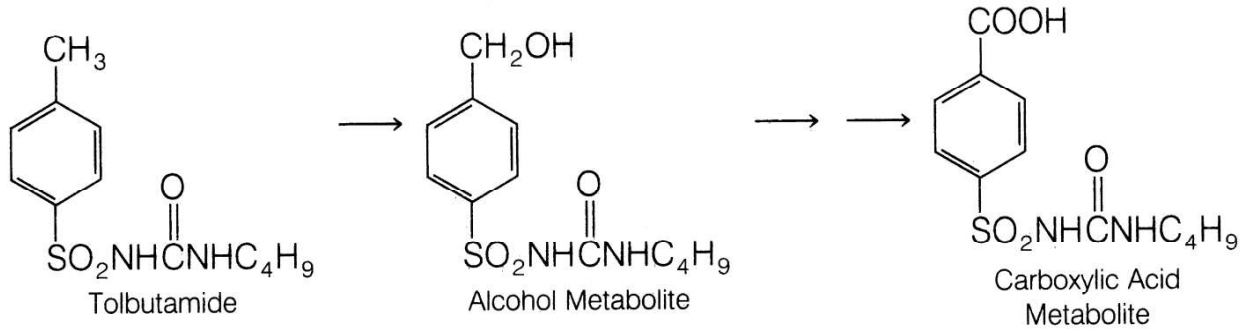
3. Oxidation at Benzyl Carbon Atoms



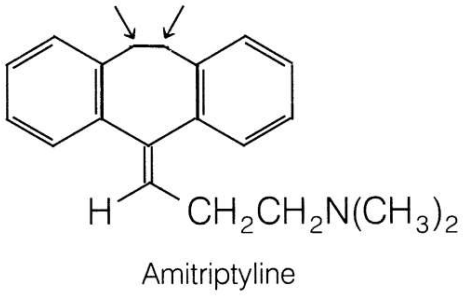
Carbon atoms attached to aromatic rings (benzylic position) are susceptible to oxidation, thereby forming the corresponding alcohol (or carbinol) metabolite.
Primary alcohol metabolites are often oxidized further to aldehydes and carboxylic acids

(-CH_2OH ⟶ -CHO → -COOH), and secondary alcohols are converted to ketones by soluble alcohol and aldehyde dehydrogenases. Alternatively, the alcohol may be conjugated directly with glucuronic acid.

E.g. the benzylic carbon atom present in the oral hypoglycemic agent tolbutamide is oxidized extensively to the corresponding alcohol and carboxylic acid. Both metabolites have been isolated from human urine.

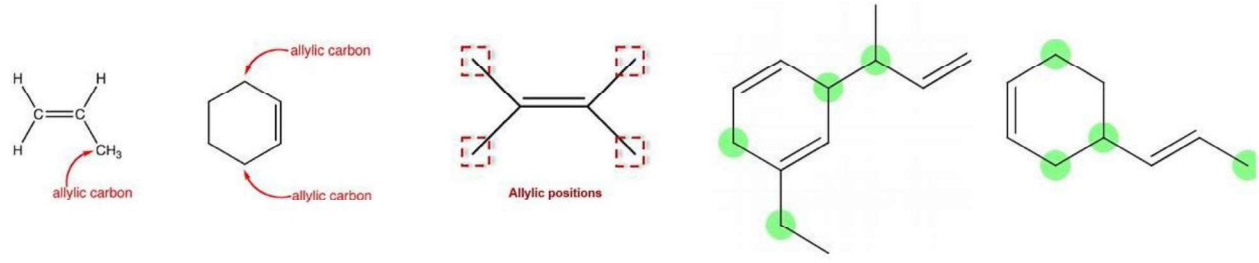


E.g. Amitriptyline



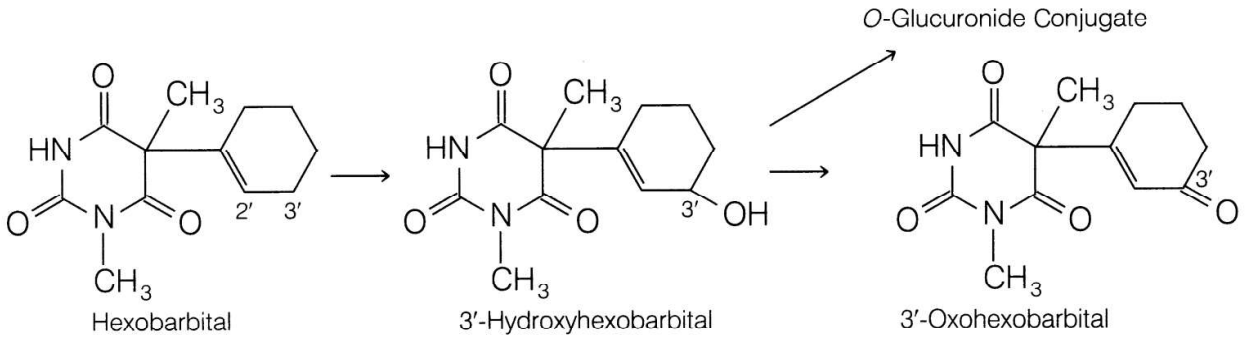
4. Oxidation at Allylic Carbon Atoms

Microsomal hydroxylation at allylic carbon atoms is commonly observed in drug metabolism.



Examples of allylic oxidation include the sedative-hypnotic hexobarbital.

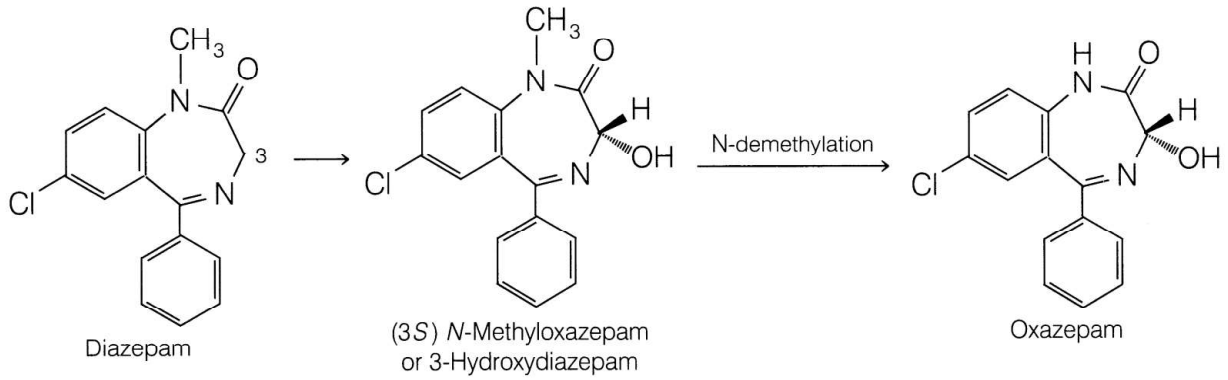
The 3′-hydroxylated metabolite formed from hexobarbital is susceptible to glucuronide conjugation as well as further oxid



ation to the 3′-oxo compound.

5. Oxidation at Carbon Atoms α to Carbonyls and Imines

The mixed-function oxidase system also oxidizes carbon atoms adjacent (i.e., α) to carbonyl and imino functionalities. An important class of drugs undergoing this type of oxidation is the benzodiazepines. Example diazepam (Valium)**.**



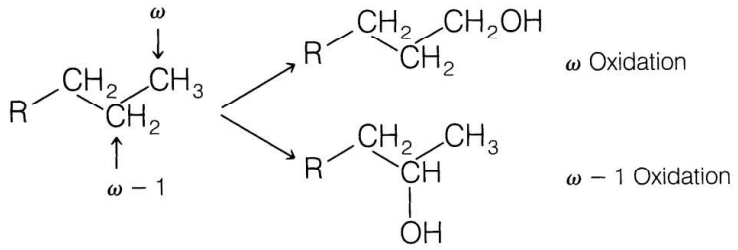
6. Oxidation of Aliphatic and Alicyclic Carbon Atom

Compounds contain straight or branched chain undergoes 2 types of oxidation:

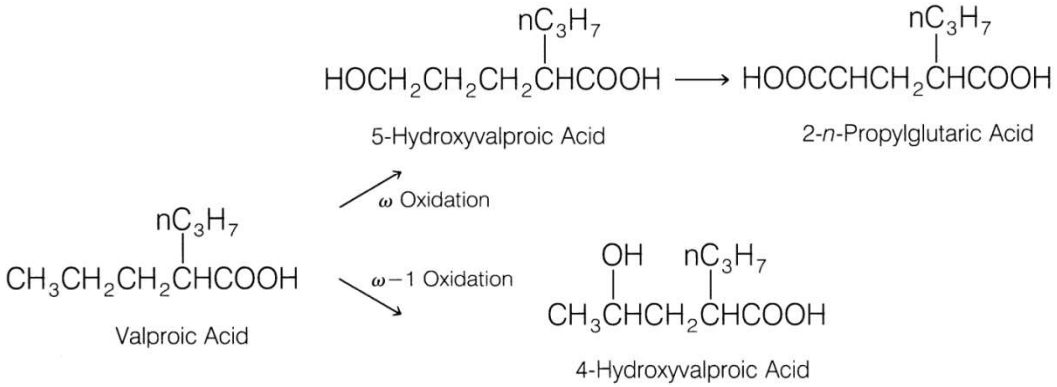
●oxidation takes place at terminal methyl group (ꝺ-oxidation).

●oxidation takes place at carbon atom before the last carbon (ꝺ-1oxidation)

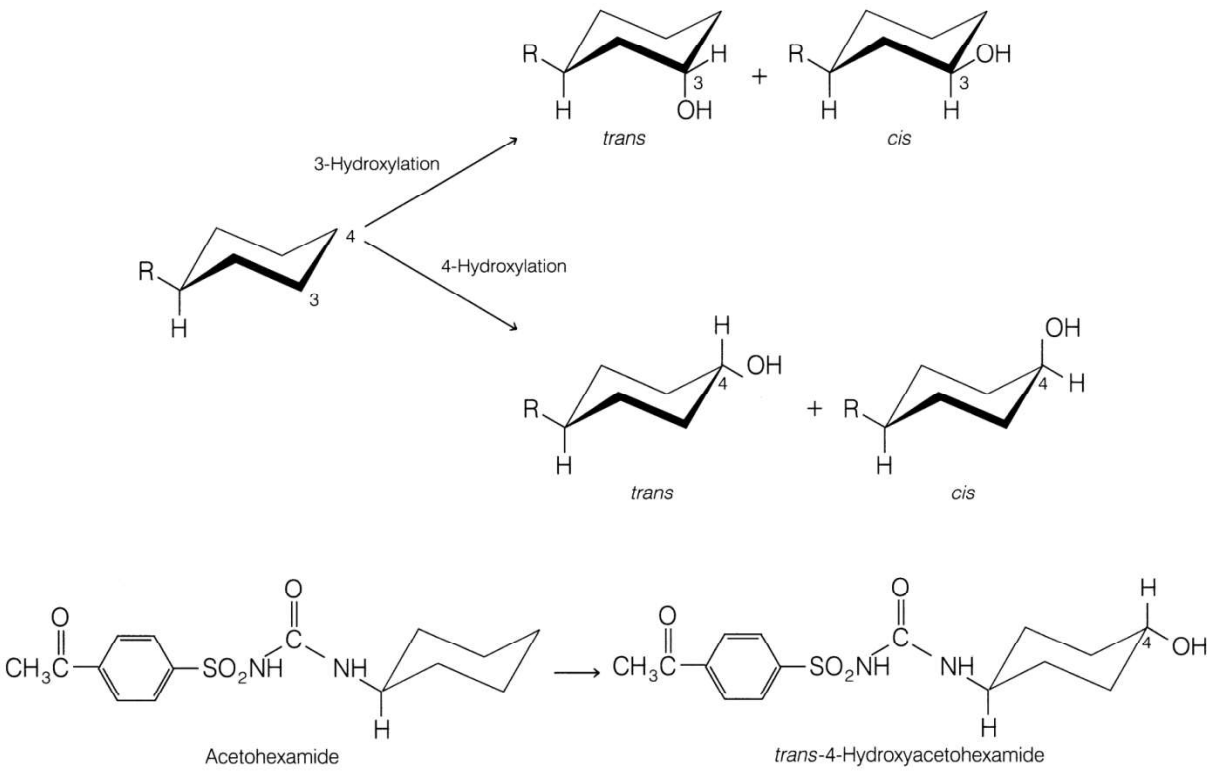
The initial alcohol metabolites formed from these enzymatic ω and ω-1 oxidations are susceptible to further oxidation to yield aldehyde, ketones, or carboxylic acids. Alternatively, the alcohol metabolites may undergo glucuronide conjugation.



E.g. the antiepileptic agent valproic acid (Depakene) undergoes both ω and ω-1 oxidation to the 5-hydroxy and 4-hydroxy metabolites, respectively. Further oxidation of the 5-hydroxy metabolite yields 2-n-propylglutaric acid.



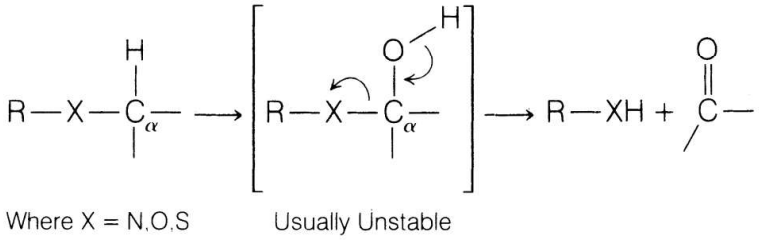
The cyclohexyl group is commonly found in many medicinal agents, and is also susceptible to mixed-function oxidation (alicyclic hydroxylation).114,115 Enzymatic introduction of a hydroxyl group into a monosubstituted cyclohexane ring generally occurs at C-3 or C-4 and can lead to cis and trans conformational stereoisomers, as shown in the following scheme. E.g. the oral hypoglycemic agent acetohexamide.



7. Oxidation Involving Carbon–Heteroatom Systems

Nitrogen and oxygen functionalities are commonly found in most drugs and foreign compounds; sulfur functionalities occur only occasionally. Metabolic oxidation of carbon–nitrogen, carbon–oxygen, and carbon–sulfur systems principally involves two basic types of biotransformation processes:

1. Hydroxylation of the -carbon atom attached directly to the heteroatom (N, O, S). The resulting intermediate is often unstable and decomposes with the cleavage of the carbon–heteroatom bond. Oxidative N-, O-, and S-dealkylation as well as oxidative deamination reactions fall under this mechanistic pathway.



2. Hydroxylation or oxidation of the heteroatom (N, S only, e.g., N-hydroxylation, N-oxide formation, sulfoxide, and sulfone formation).

a. Oxidation involving carbon–nitrogen systems

Metabolism of nitrogen functionalities (e.g., amines, amides) is important because such functional groups are found in many natural products (e.g., morphine, cocaine, nicotine) and in numerous important drugs (e.g., phenothiazines, antihistamines, tricyclic antidepressants, β-adrenergic agents, sympathomimetic phenylethylamines, benzodiazepines).

Nitrogen-containing compounds are divided into three basic classes:

1. Aliphatic (primary, secondary, and tertiary) and alicyclic (secondary and tertiary) amines.

2. Aromatic and heterocyclic nitrogen compounds.

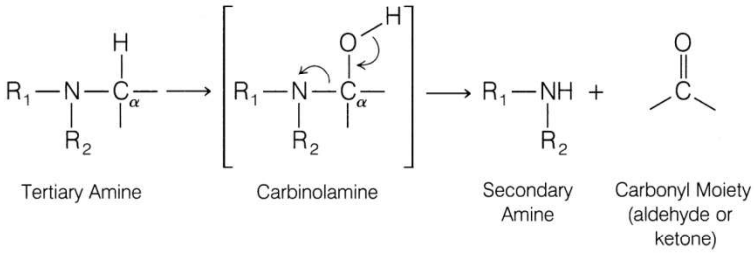
3. Amides.

1. Tertiary Aliphatic and Alicyclic Amines

a. Oxidative N-dealkylation

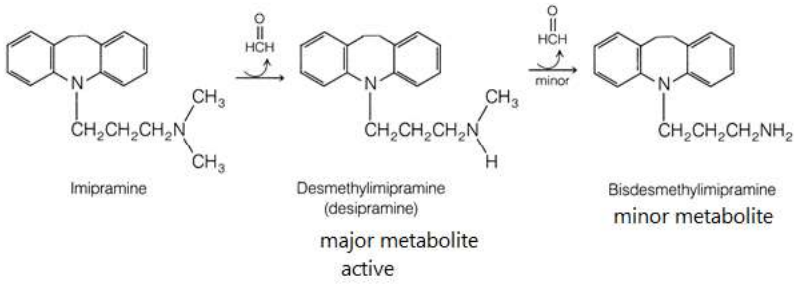
It is the oxidative removal of alkyl groups (particularly methyl groups) from tertiary aliphatic and alicyclic amines which is carried out by hepatic CYP mixed-function oxidase enzymes.

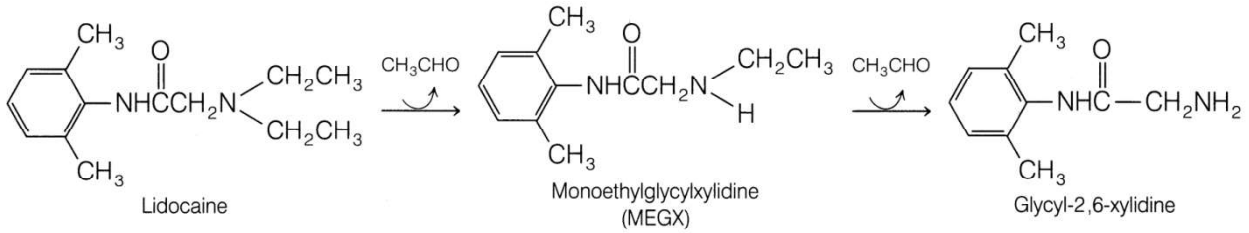
The initial step involves α-carbon hydroxylation to form a carbinolamine intermediate, which is unstable and undergoes spontaneous heterolytic cleavage of the C–N bond to give a secondary amine and a carbonyl moiety (aldehyde or ketone).



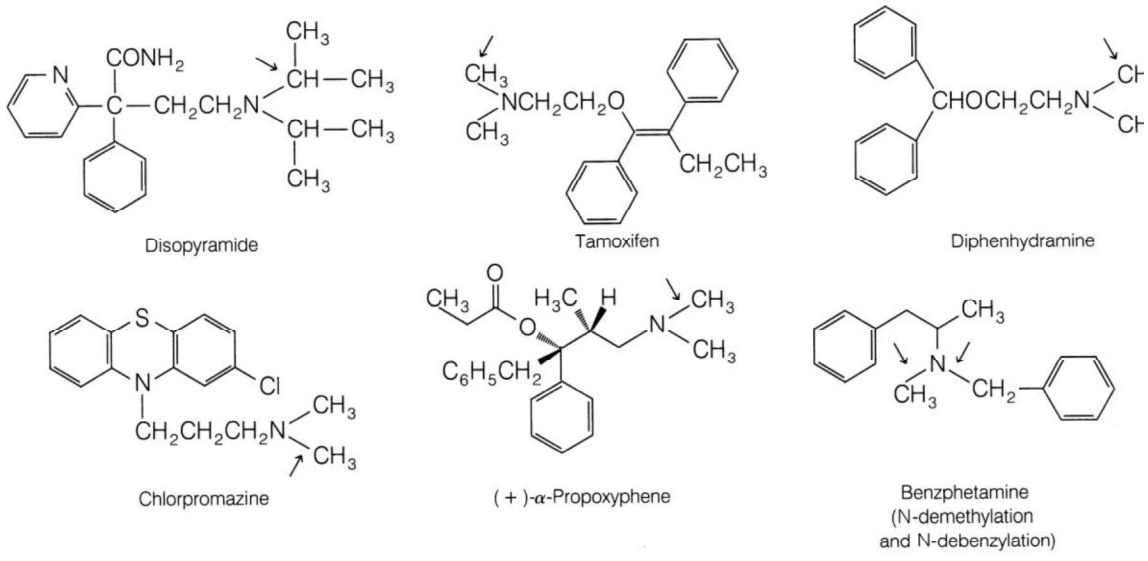
In general, small alkyl groups, such as methyl, ethyl, and isopropyl, are removed rapidly. N-dealkylation of the t-butyl group is not possible by the carbinolamine pathway because α-carbon hydroxylation cannot occur. E.g. the N-t-butyl group present in many β-adrenergic antagonists, such as terbutaline and salbutamol, remains intact and does not appear to undergo any significant metabolism.

The first alkyl group from a tertiary amine is removed more rapidly than the second alkyl group. In some instances, bisdealkylation of the tertiary aliphatic amine to the corresponding primary aliphatic amine occurs very slowly. E.g.imipramine (Tofranil ®) and lidocaine.

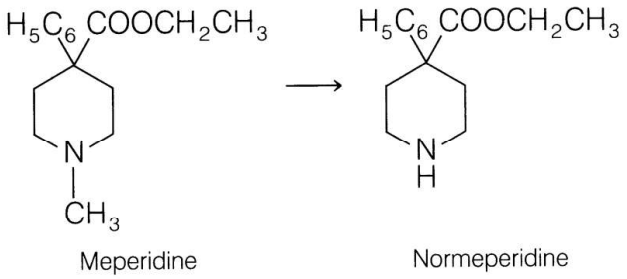




Other examples of tertiary aliphatic amine drugs which are metabolized principally by oxidative N-dealkylation:



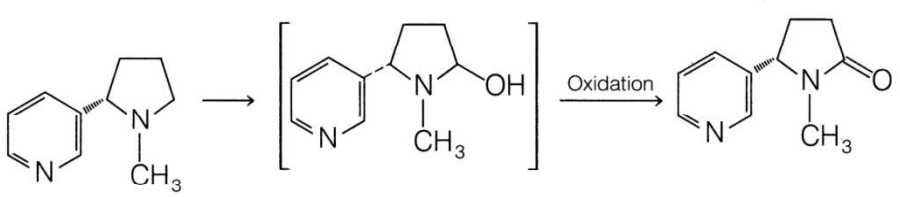
Like their aliphatic counterparts, alicyclic tertiary amines are susceptible to oxidative N-dealkylation reactions. For example, the analgesic drug meperidine.



Meperidine Normeperidine

b. Formation of lactam metabolites

Alicyclic tertiary amines often generate lactam metabolites by α-carbon hydroxylation reactions at the ring carbon atom α to the nitrogen which further oxidized to lactam metabolites. E.g. nicotine undergoes this reaction.



Nicotine Carbinolamine Cotinine

c. N-oxidation

Tertiary amines possibly undergo N-oxidation leading to formation of the active N-oxide metabolite. E.g. the tricyclic antidepressants imipramine.

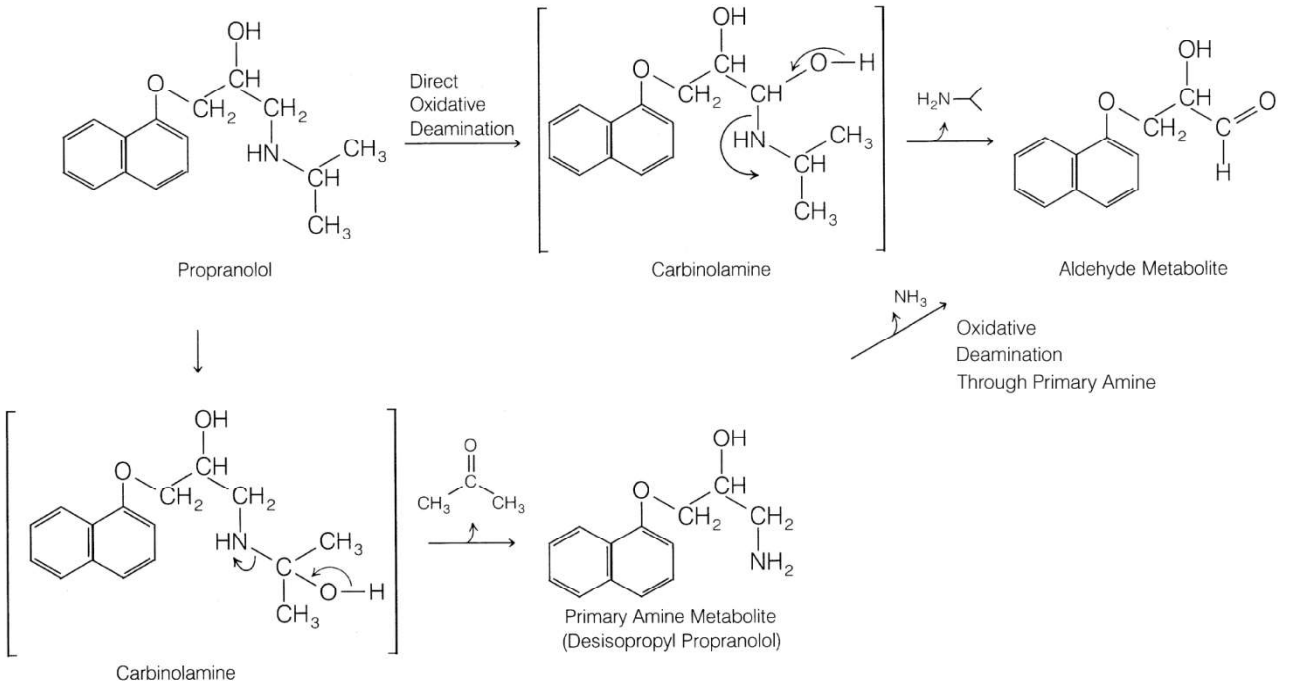
2. Secondary and Primary Amines.

a. Secondary amines (either parent compounds or metabolites) are susceptible to

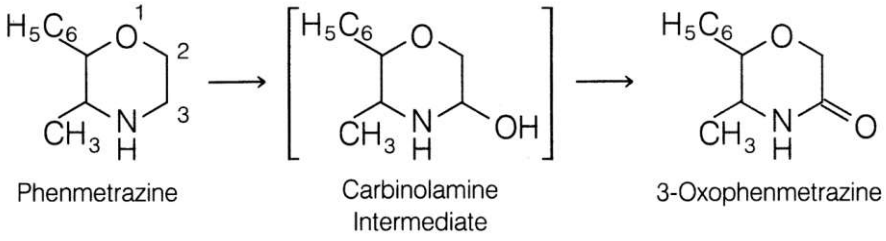
- oxidative N-dealkylation,
- oxidative deamination, and
- N-oxidation reactions.

- As in tertiary amines, N-dealkylation of secondary amines proceeds by the carbinolamine pathway. Dealkylation of secondary amines gives rise to the corresponding **primary amine metabolite**. For example, the β-adrenergic blocker propranolol.
- The primary amine metabolites formed from oxidative dealkylation are susceptible to oxidative deamination. This process is similar to N-dealkylation, in that it involves an initial α-carbon hydroxylation reaction to form a carbinolamine intermediate, which then undergoes subsequent carbon–nitrogen cleavage to the **carbonyl metabolite** and **ammonia**.

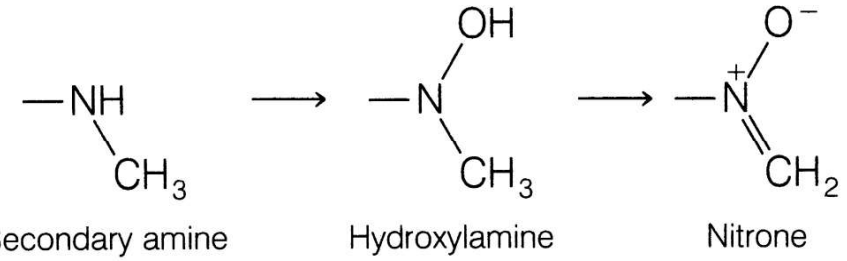
- Direct deamination of the secondary amine (ex. Propranolol) also has occurred. In addition to undergoing deamination after oxidative N-dealkylation, propranolol can undergo a direct oxidative deamination reaction (also by α-carbon hydroxylation) to yield the **aldehyde metabolite** and **alkylamine** (isopropylamine).

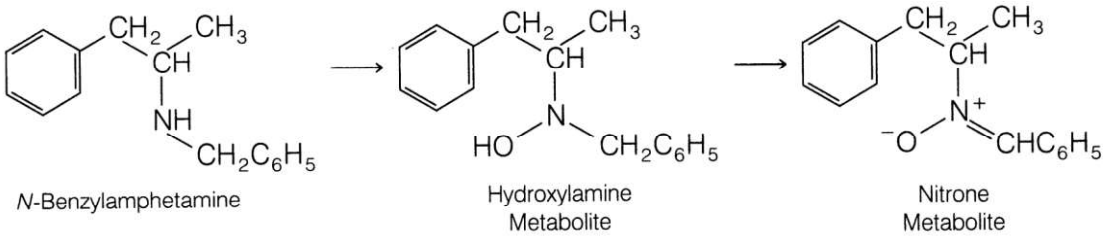


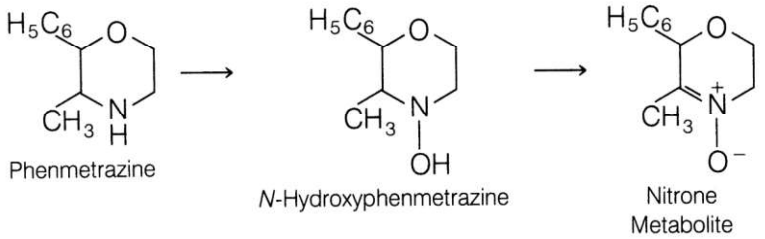
- Some secondary alicyclic amines, like their tertiary amine analogs, are metabolized to their corresponding **lactam derivatives**. For example, the anorectic agent phenmetrazine.



- Metabolic N-oxidation of secondary aliphatic and alicyclic amines leads to several N-oxygenated products. N-hydroxylation of secondary amines generates the corresponding N-hydroxylamine metabolites. These hydroxylamine products are susceptible to further oxidation (either spontaneous or enzymatic) to the corresponding nitrone derivatives. E.g. N-benzylamphetamine and phenmetrazine.

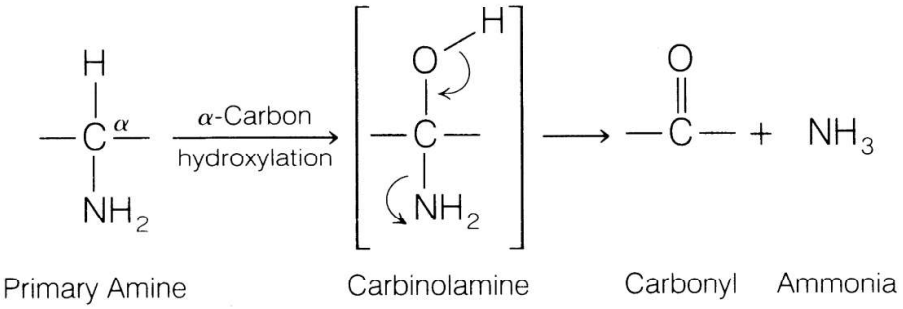




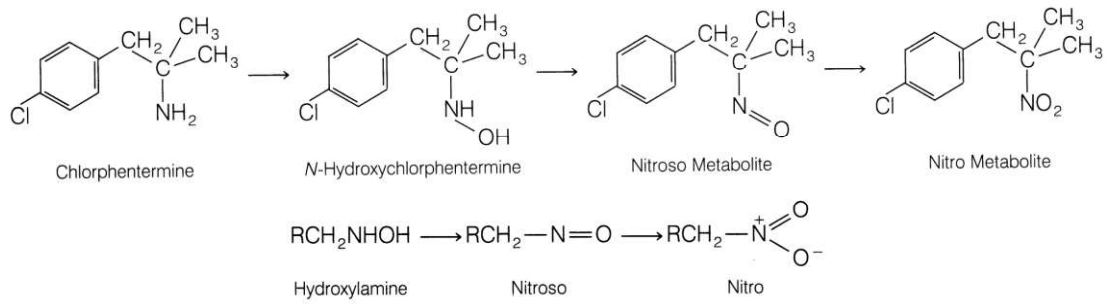


b. Primary aliphatic amines (whether parent drugs or metabolites) are biotransformed by:

- Oxidative deamination (through the carbinolamine pathway) by CYP which leads to the formation of carbonyl metabolites and ammonia.



- N-oxidation which leads to the formation of N-hydroxyl amine metabolites which are susceptible to further oxidation to yield other *N*-oxygenated products like nitroso (N=O) and the nitro (nitrogen dioxide).

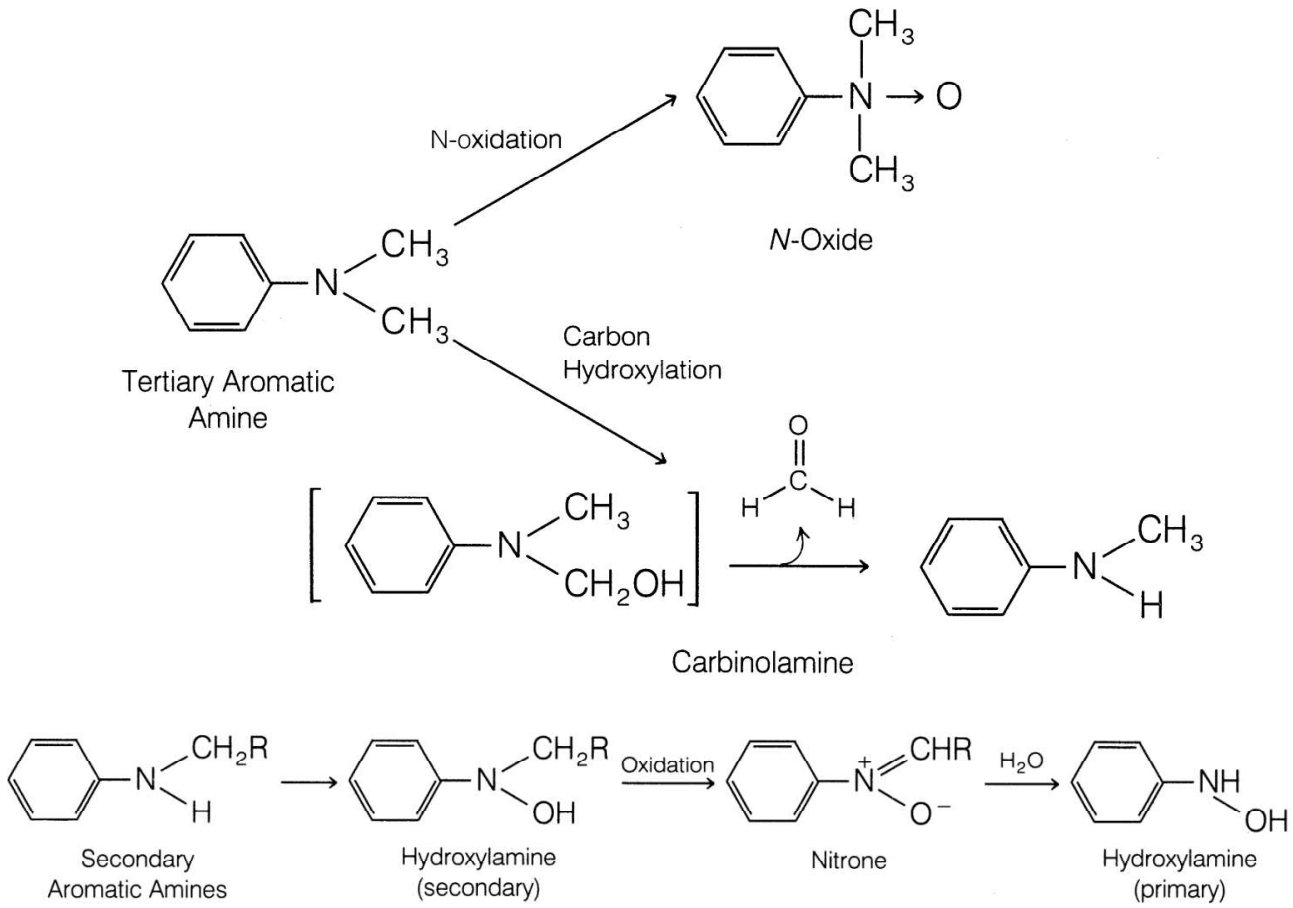


Oxidative deamination of primary amines

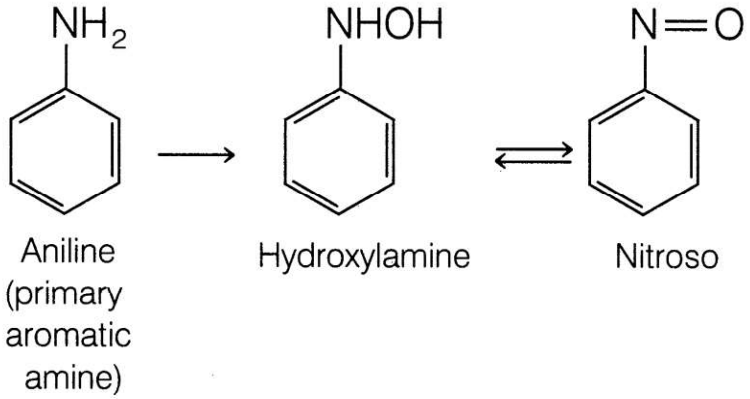
3. Tertiary aromatic amines

tertiary aromatic amines (such as *N*,*N*-dimethylaniline) and secondary aromatic amines can undergo oxidative *N*-dealkylation as well as *N*-oxide formation take place. Further

oxidation of the *N*-hydroxylamine leads to nitrone products, which in turn may be hydrolyzed to primary hydroxylamines.

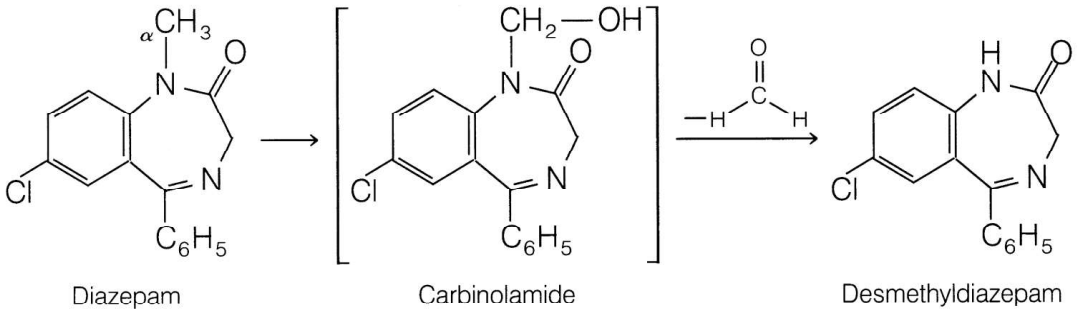


N-oxidation of primary aromatic amines generates the N-hydroxylamine metabolite. One such case is aniline, which is metabolized to the corresponding N-hydroxy product.223 Oxidation of the hydroxylamine derivative to the nitroso derivative also can occur.

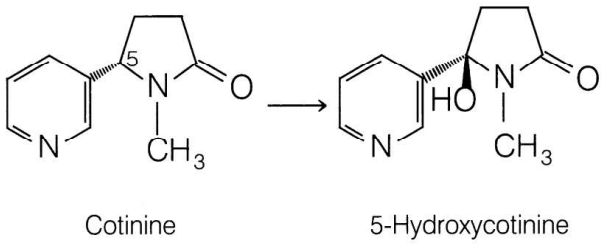


4. Amides

Amide functionalities are susceptible to oxidative carbon–nitrogen bond cleavage (via -carbon hydroxylation) and N-hydroxylation reactions. Oxidative dealkylation proceeds via an initially formed carbinolamide, which is unstable and fragments to form the N-dealkylated product. For example, diazepam undergoes extensive N-demethylation to the pharmacologically active metabolite desmethyldiazepam.

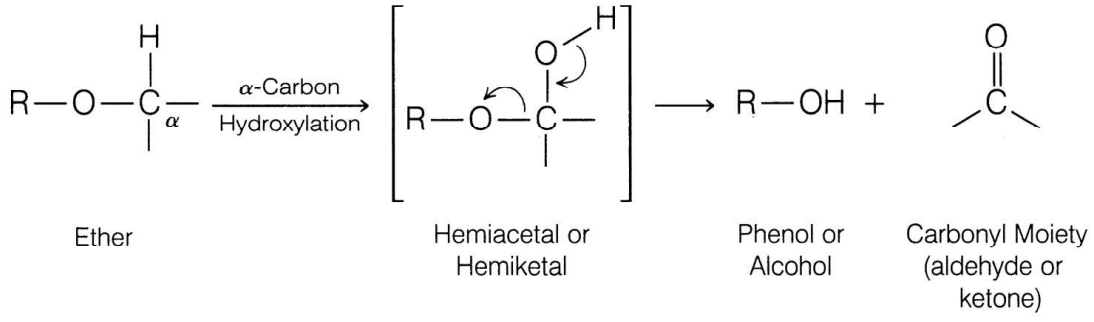


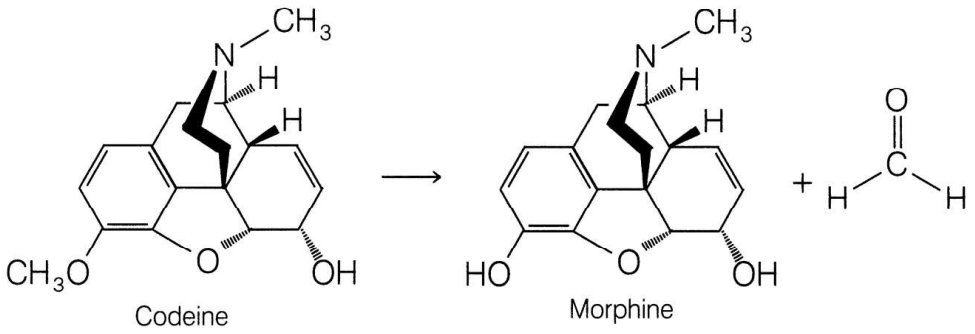
In the cyclic amides or lactams, hydroxylation of the alicyclic carbon to the nitrogen atom also leads to carbinolamides. An example of this pathway is the conversion of cotinine to 5-hydroxycotinine.



b. Oxidation Involving Carbon-Oxygen (ethers)

The biotransformation of ethers involves an initial α-carbon hydroxylation to form either hemiacetal or hemiketal, which undergoes spontaneous carbon-oxygen bond cleavage to yield the dealkylated oxygen species (phenol or alcohol) and a carbonyl moiety (aldehyde or ketone).

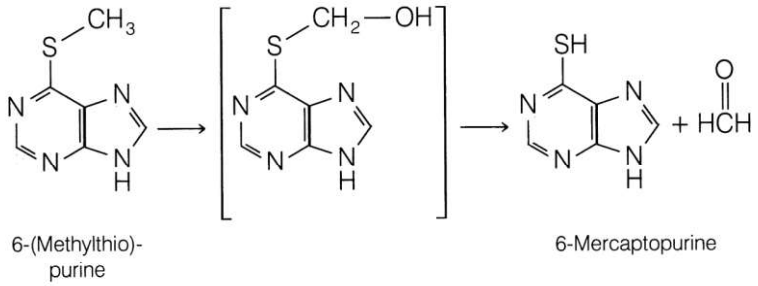




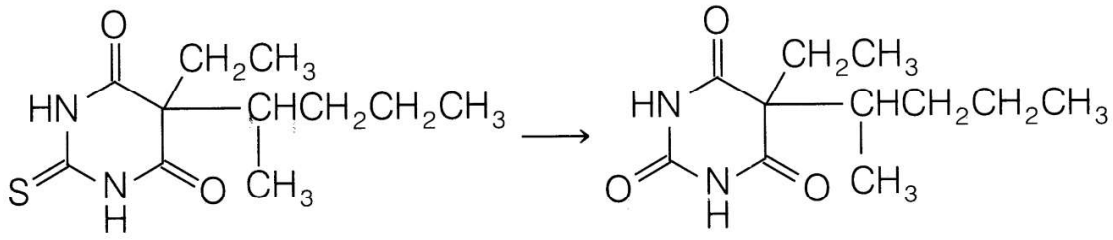
c. Oxidation Involving Carbon-Sulfur

Several drugs containing Carbon-Sulfur functional group are susceptible to

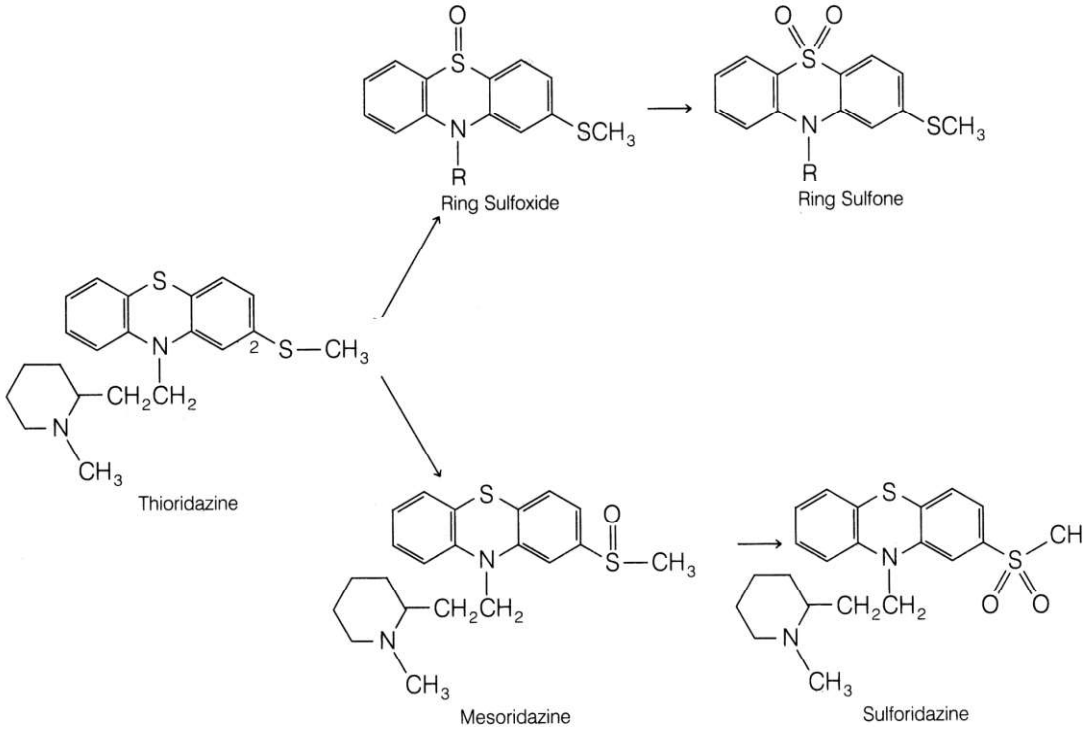
1. S-dealkylation: Similar to N- and O-dealkylation and involves oxidative carbon-sulfur bond cleavage.



2. Desulfuration: Oxidative conversion of carbon-sulfur double bonds (C=S) (thiono) to the corresponding carbon-oxygen double bond (C=O) is called desulfuration.



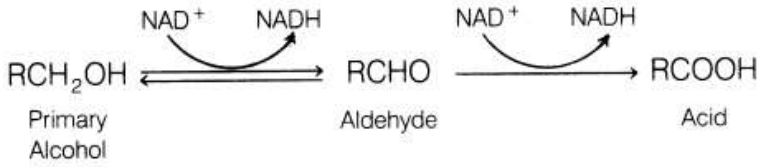
3. S-oxidation: S-oxidation yields the corresponding sulfoxide derivatives. Sulfoxide drugs/metabolites may further oxidised to sulfones (-SO2).



Oxidation of Alcohols and Aldehydes

Many oxidative processes (e.g., benzylic, allylic, alicyclic, or aliphatic hydroxylation) generate alcohol or carbinol metabolites as intermediate products. If not conjugated, these alcohol products are further oxidized to aldehydes (if primary alcohols) or to ketones (if secondary alcohols).

Aldehyde metabolites resulting from oxidation of primary alcohols or from oxidative deamination of primary aliphatic amines often undergo oxidation to generate polar carboxylic acid derivatives.



The bioconversion of alcohols to aldehydes and ketones is catalyzed by soluble alcohol dehydrogenases present in the liver and other tissues.

Oxidation of secondary alcohol to ketones is NOT often important as it reduces back to secondary alcohol. Secondary alcohol group being more polar and functionalized, is more likely to be conjugated than the ketone moiety.

Other Oxidative Biotransformation Pathways

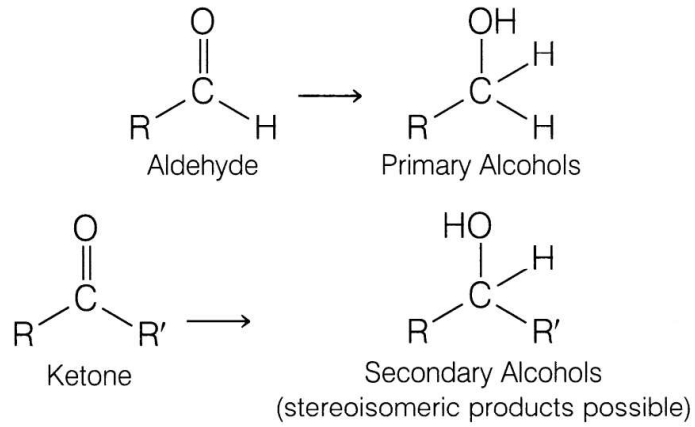
1. Oxidative aromatization reactions ex, the progesterone derivative norgestrel.
2. Oxidative dehalogenation reactions ex, volatile anesthetic agent halothane.

Reduction Reactions

Reductive process play an important role in the metabolism of many compounds containing carbonyl, nitro and azo groups. Bioreduction of carbonyl compounds generates alcohol derivatives, whereas nitro and azo reductions lead to amino derivatives. The hydroxyl and amino moieties of the metabolites are much more susceptible to conjugation than the functional groups of the parent compounds. Therefore, reductive processes, as such, facilitate drug elimination.

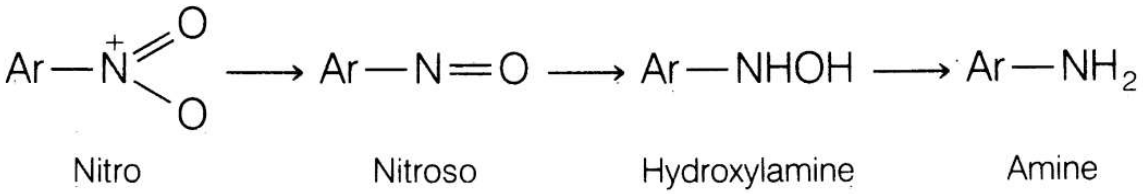
Reduction of Aldehydes and Ketones

- Aldehydes reduced to primary alcohols.
- Ketones reduced to secondary alcohols.
- Reactions mediated by *Aldo-Keto reductase* enzymes
- Bioreduction of ketones often leads to the creation of an asymetric center and thereby produces two possible stereoisomeric alcohols.
- One of the stereoisomer may preferentially form predominantly over other stereoisomer and thus shows product stereo selectivity in drug metabolism.

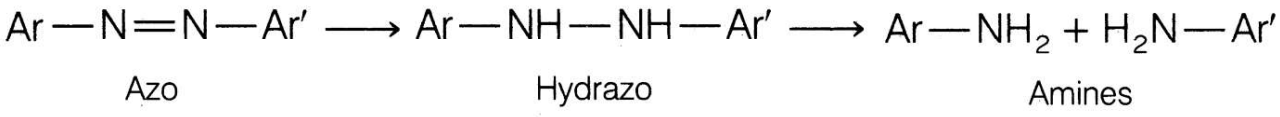


Reduction of Nitro and Azo Compounds

- Bioreduction of aromatic nitro and azo compounds leads to aromatic primary amine metabolites.
- Aromatic nitro compounds are reduced initially to the nitroso and ydroxylamine intermediates that subsequently further reduced to amine.



- Azo reduction proceed via hydrazo intermediate (-NH-NH-) that subsequently cleaved reductively to yield the corresponding amines.

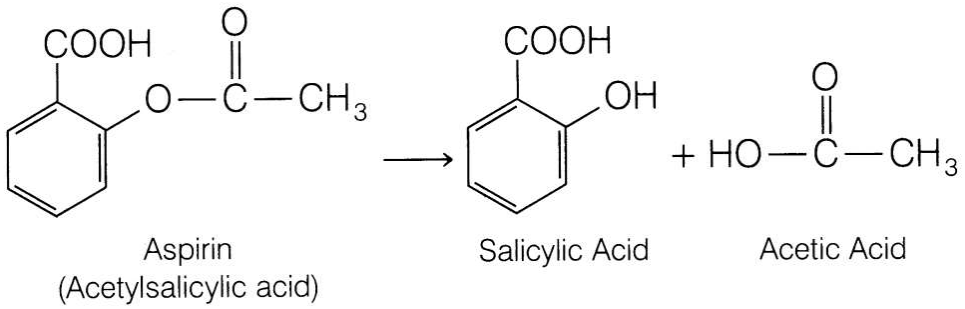


Phase I Hydrolytic reaction:

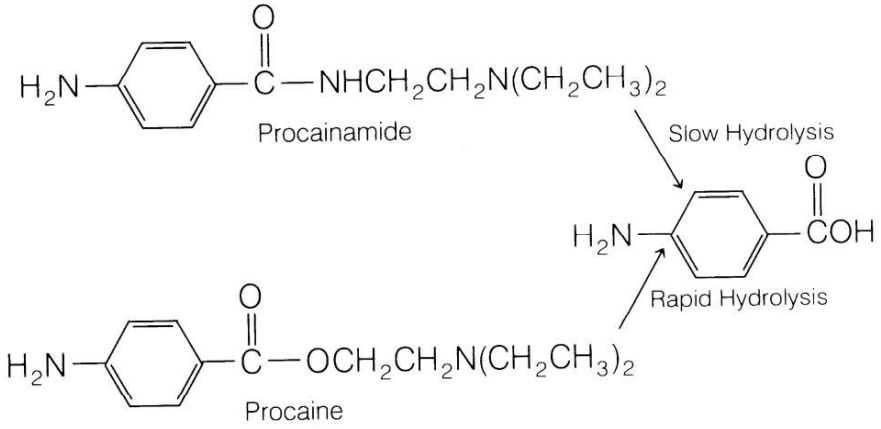
Hydrolysis of Esters and Amides

The metabolism of ester and amide linkages in many drugs is catalyzed by hydrolytic enzymes present in various tissues and in plasma. The metabolic products formed (carboxylic acids, alcohols, phenols, and amines) generally are polar and functionally more susceptible to conjugation and excretion than the parent ester or amide drugs. The enzymes carrying out ester hydrolysis include several nonspecific esterases found in the liver, kidney, and intestine as well as the pseudocholinesterases present in plasma. Amide hydrolysis appears to be mediated by liver microsomal amidases, esterases, and deacylases.

Hydrolysis is a major biotransformation pathway for drugs containing an ester functionality. This is because of the relative ease of hydrolyzing the ester linkage. A classic example of ester hydrolysis is the metabolic conversion of aspirin (acetylsalicylic acid) to salicylic acid.



Amides are hydrolyzed slowly in comparison to esters.337 Consequently, hydrolysis of the amide bond of procainamide is relatively slow compared with hydrolysis of the ester linkage in procaine.



Miscellaneous Hydrolytic Reactions

1. Hydrolysis of recombinant human peptide drugs and hormones at the N- or C-terminal amino acids by carboxypeptidase and aminopeptidase and proteases in blood and other tissues. Examples of peptides or protein hormones undergoing hydrolysis include human insulin, growth hormone (GH), prolactin, parathyroid hormone (PTH) Examples of peptides or protein hormones undergoing hydrolysis include human insulin, growth hormone (GH), prolactin and parathyroid hormone (PTH).
2. The hydrolysis of phosphate esters (e.g., diethylstilbestrol diphosphate), sulfonylureas, cardiac glycosides, carbamate esters, and organophosphate compounds.
3. Glucuronide and sulfate conjugates also can undergo hydrolytic cleavage by β-glucuronidase and sulfatase enzymes.