Phase II (Conjugation) Reactions.

Introduction

- Phase I or functionalization reactions do not always produce hydrophilic or pharmacologically inactive metabolites and nontoxic.
- Various phase II or **conjugation reactions**, however, can convert these metabolites to more polar and water-soluble products.
- Many conjugative enzymes accomplish this objective by attaching small, polar, and ionizable endogenous molecules, such as glucuronic acid, sulfate, glycine, and glutamine, to the phase I metabolite or parent xenobiotic.
- The resulting conjugated products are relatively water soluble and readily excretable. In addition, they generally are biologically inactive and nontoxic.
- Other phase II reactions, such as **methylation** and **acetylation,** do not generally increase water solubility but mainly serve to terminate or attenuate pharmacological activity.
- The role of **GSH** is to combine with chemically reactive compounds to prevent damage to important biomacromolecules, such as DNA, RNA, and proteins.
- Thus, phase II reactions can be regarded as truly **detoxifying pathways** in drug metabolism, with a **few exceptions.**
- A distinguishing feature of most phase II reactions is that the conjugating group (glucuronic acid, sulfate, methyl, and acetyl) is activated initially in the form of a coenzyme before transfer or attachment of the group to the accepting substrate by the appropriate transferase enzyme.
- In other cases, such as glycine and glutamine conjugation, the substrate (amino acid) is activated initially.
    - All of the conjugating groups need to be activated to react with drug molecule
    - The activation process is carried out by enzyme using two main approaches:

Reactions	Enzymes	Drug Functional Groups	Activated Coenzyme
Glucuronidation	UDP-glucuronyltransferase	OH, SH, COOH, NH_2	Uridine diphosphate glucuronic acid (UDPGA).
Sulfation	Sulfotransferase	OH, NH_2, SH	3-Phosphoadenosine5—phosphosulfate(PAPS)
Methylayion	Methyl transferase	OH, NH_2, SH	S-Adenosinemethaionine(SAM)
Acetylation	Acetyl transferase	OH, NH_2	Acetyl-CoA
Amino acid conjugation	Acetylcoenzyme A (ATP)	COOH	Substrate derivative of AMP&CoASH
Glutathione conjugation	Glutathione-S-transferase	Epoxide, organic halides	No Activation

Phase II reactions include:

1. Conjugation; it includes:

A. Glucuronic acid (GUA) conjugation.

B. Sulfate conjugation.

C. Amino acid(a.a.) conjugation (glycine, and glutamine).

D. Glutathione (GSH) reaction.

2. Acetylation.

3. Methylation.

- Other minor conjugative pathways are conjugation with glycosides, phosphate, and other amino acids and conversion of cyanide to thiocyanate.

1. Conjugation

A. Glucuronic Acid (GUA) Conjugation

- Glucuronidation is the major phase II metabolic path way for drugs, xenobiotics and endogenous compounds for the following reasons:

a) A readily available supply of D-glucuronic acid (derived from D-glucose),

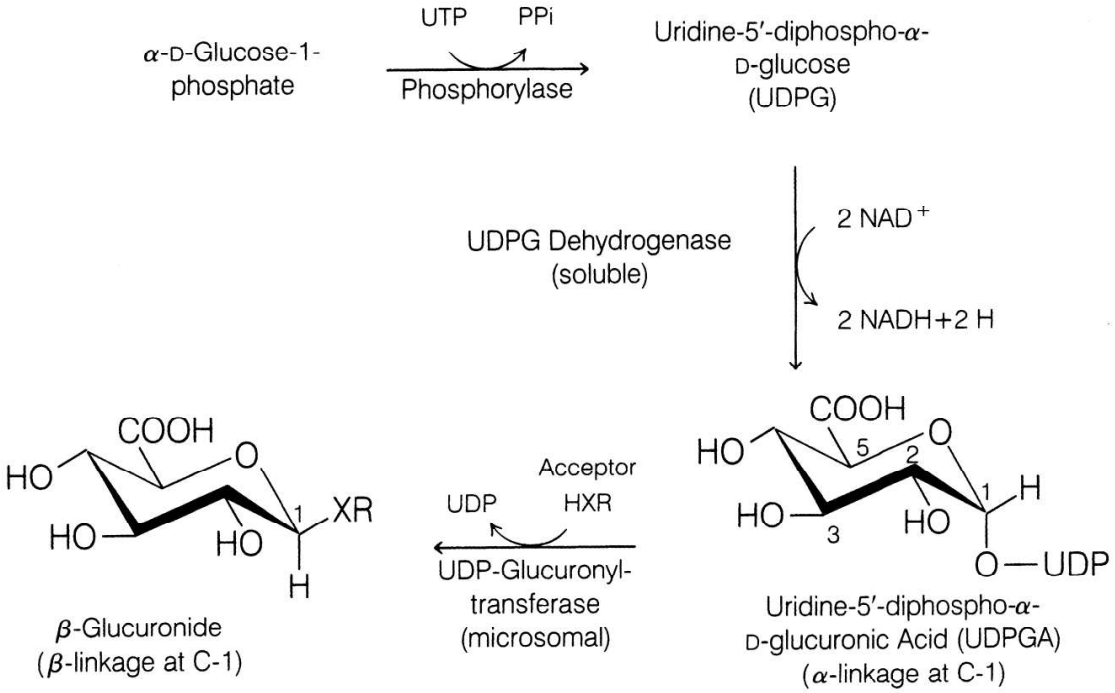
b) Numerous functional groups that can combine enzymatically with glucuronic acid,

c) The glucuronyl moiety (with its ionized carboxylate [pKa 3.2] and polar Hydroxyl groups), which, when attached to xenobiotic substrates greatly increases the water solubility of the conjugated product.

- Formation of β-glucuronides involves two steps:

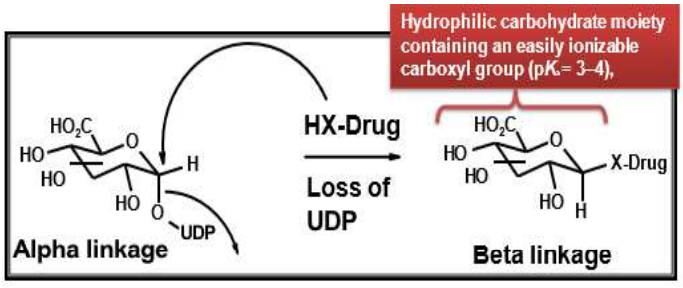
a. Synthesis of an activated coenzyme, uridine-5-diphospho-α-D-glucuronic acid (UDPGA),

b. Subsequent transfer of the glucuronyl group from UDPGA to an appropriate substrate. This step is catalyzed by microsomal enzymes called *UDP-glucuronyltransferases*.



Formation of UDPGA and β-glucuronide conjugates. The synthesis of the coenzyme UDPGA uses α-D-glucose- 1-phosphate as its initial precursor

- All glucuronide conjugates have the β-configuration or β-linkage at C-1 (hence, the term β-*glucuronides*).
- In contrast, the coenzyme UDPGA has an α-linkage, the above enzymatic transfer step, is nucleophilic displacement of the α-linked UDP moiety from UDPGA by the substrate RXH (X = O, N, S &C) proceeds with complete inversion of configuration at C-1 to give the β-glucuronide (SN^2).



- Diglucuronide conjugates do not usually occur, because glucuronidation of one functional group is sufficient for the excretion of the conjugated metabolite (in urine).
- β-Glucuronides are classified according to the heteroatom attached to the C-1 atom of the glucuronyl group. into:
  i). O-β-Glucuronides.

  ii). N-β-Glucuronides.

  iii). S-β-Glucuronides.

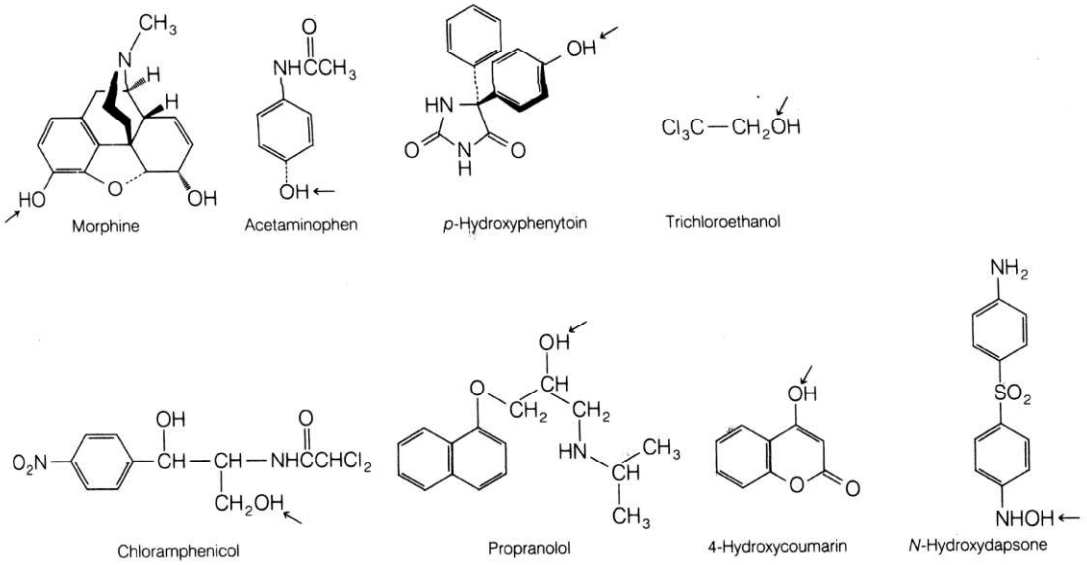
  iv). C-β-Glucuronide.

- The functional groups undergoing glucuronidation in drug metabolism are hydroxy (phenolic and alcoholic hydroxyls) and carboxy, amino, thiol and even Carbone in some cases as illustrated below
- Several endogenous substrates, e.g., bilirubin and steroids are eliminated as glucuronide conjugates, which are excreted primarily in the urine.
- Due to the large size of some glucuronide conjugates (>300 Da), they are excreted to bile (e.g., steroids).
- However, in the intestine, the conjugates are hydrolyzed by β-glucuronidase enzymes and release drug which may be reabsorbed (entero-hepatic circulation that increases half-life of drug)

i). O-β-Glucuronides.

The most common functional groups undergoing glucuronidation in drug metabolism to form *O*-glucuronides, are hydroxy (phenolic and alcoholic hydroxyls) and carboxy.

Examples for drugs undergo O-glucuronidation: at phenolic and alcoholic hydroxyl groups as well as at the carboxylic group are illustrated below:

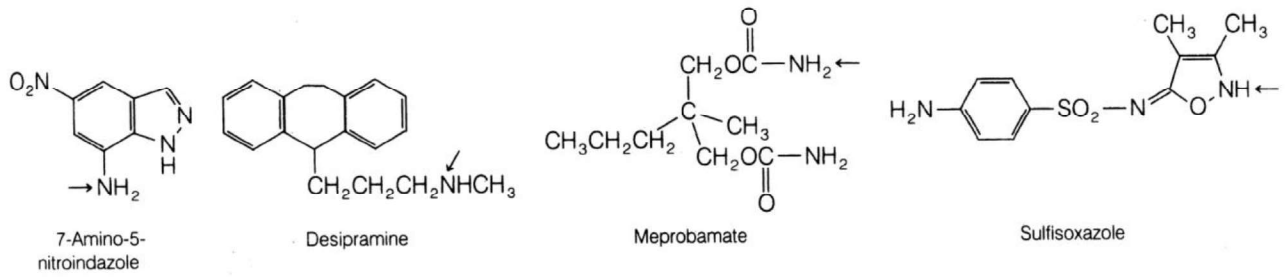


ii). N-β-Glucuronides.

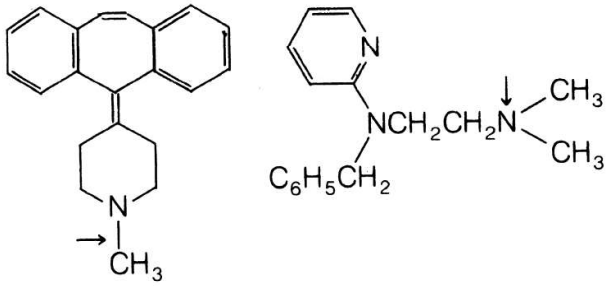
The most common functional groups undergoing glucuronidation in drug metabolism to form *N*-glucuronides, are: Aromatic & aliphatic amines, ($PhNH_2$, RNH_2, RNHR & R_3N); Amides ($RCONH_2$) & Sulfonamides (RSO_2NH_2).

Examples for drugs undergo N-glucuronidation are illustrated below:

- Glucuronidation of aromatic and aliphatic amines is generally a minor pathway in comparison with *N*-acetylation (for aromatic) or oxidative processes (e.g., oxidative deamination for aliphatic**).**



- Tertiary amines, such as the cyproheptadine quaternary ammonium glucuronide metabolites as in these examples.

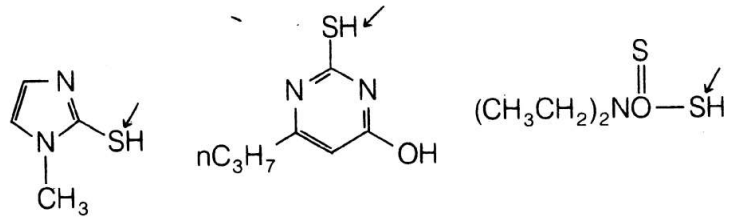


Cyproheptadine Tripelennamine

iii). S-β-Glucuronides.

This pathway is very minor metabolic pathway. The functional groups undergoing S-glucuronidation are compounds with free SH group.

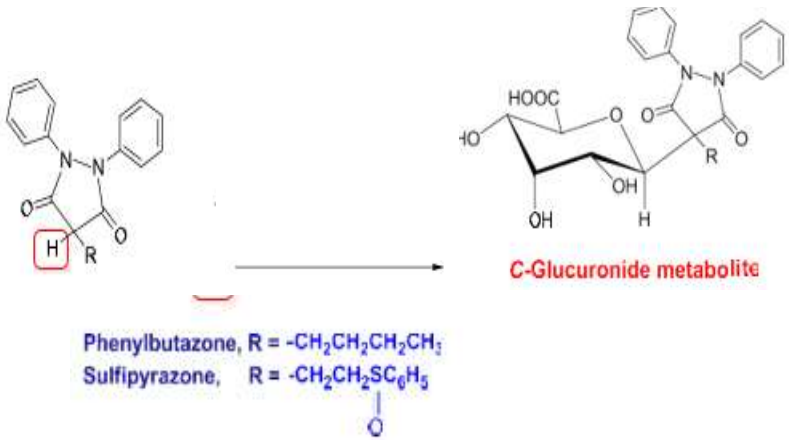
- Examples for drugs undergo S-glucuronidation are illustrated below:



Methimazole Propylthiouracil Diethylthio-carbamic Acid

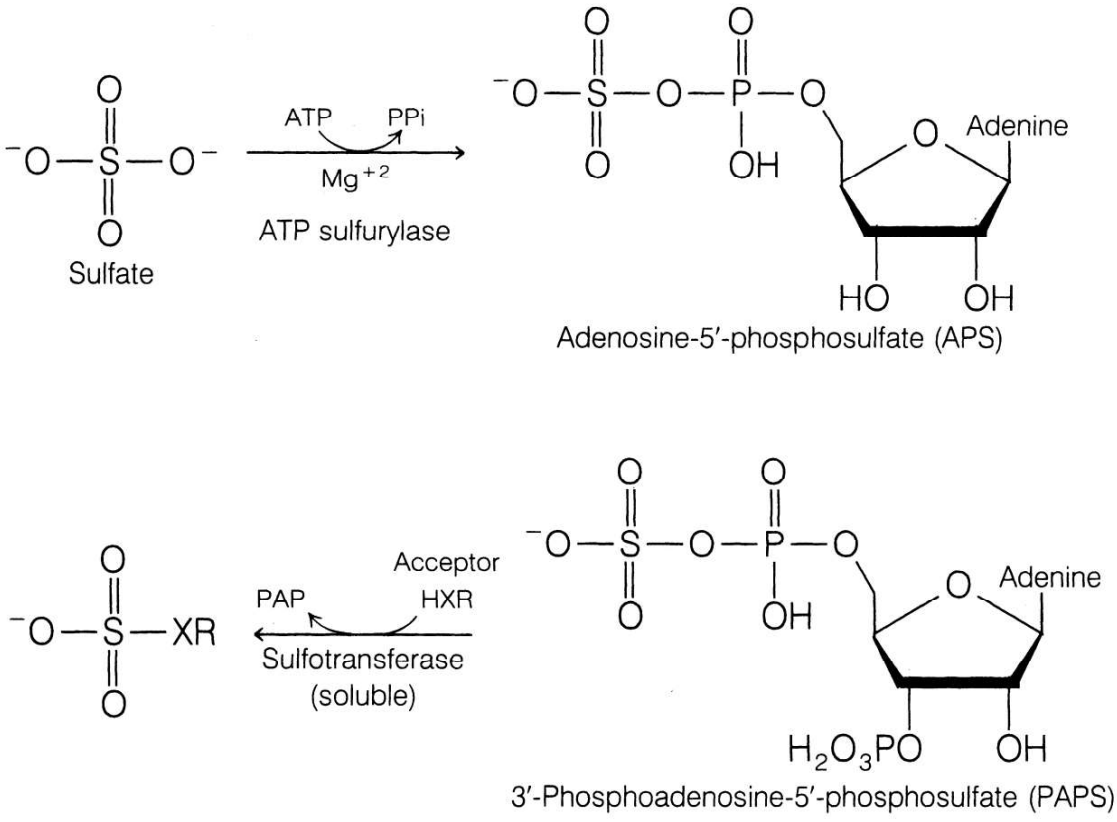
iv. C-β-Glucuronide.

This pathway is relatively rare pathway in drug metabolism as shown in the following example:



B. Sulfate (SO4) Conjugation

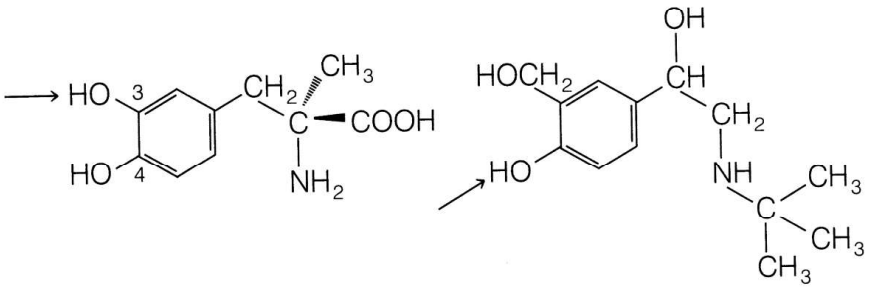
- Sulfate conjugation (Sulfation) is less frequent than glucuronide conjugation (glucuronidation).
- The most common functional groups undergoing sulfation are free OH, NH_2 & NHOH, it occurs mainly for phenols > alcohols > aromatic amines > N-hydroxyl.
- Sulfation is mainly used to conjugate endogenous compounds such as steroids, heparin, chondroitin, catecholamines, and thyroxine.
- The sulfate conjugation process involves activation of inorganic sulfate (SO_4) to the coenzyme 3′-phosphoadenosine- 5′-phosphosulfate (PAPS).
- Subsequent transfer of the sulfate group from PAPS to the accepting substrate is catalyzed by sulfotransferases.



Formation of PAPS and sulfate conjugates

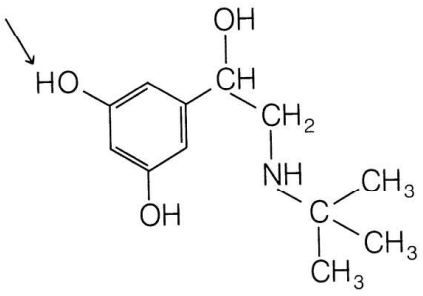
- Sulfate conjugation generally leads to water-soluble and inactive metabolites.
- However, the *O*-sulfate conjugates of some *N*-hydroxy compounds give rise to chemically reactive intermediates that are toxic.

- Phenols are the main group of substrates which undergo sulfate conjugation.
- Examples for phenol containing drugs being conjugated by sulfation include α-methyldopa, salbutamol and terbutaline as shown below.



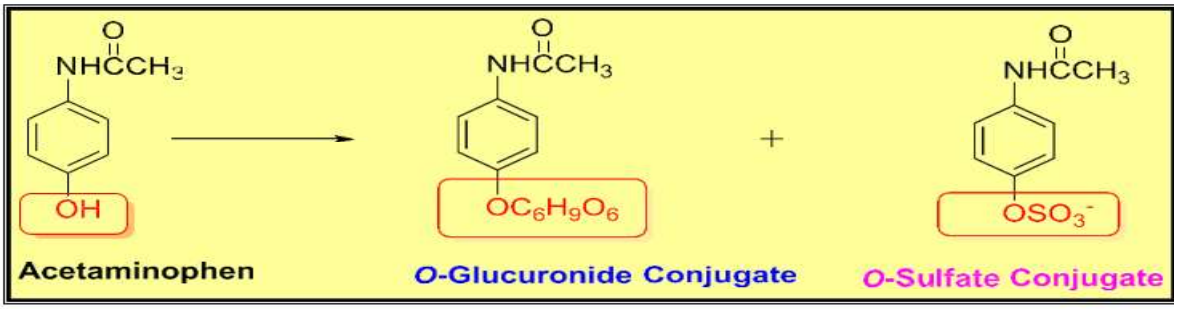
α-Methyldopa

Salbutamol
(Albuterol)



Terbutaline

- Glucuronidation of phenols is frequently a competing reaction to sulfation, the major urinary metabolite acetaminophen is the O-glucuronide conjugate, with the *O*-sulfate conjugate being formed in small amounts (minor) as shown below.
- Exception neonates and young children (ages 3–9 years) have a decreased glucuronidating capacity because of undeveloped glucuronyltransferases or low levels these enzymes. Sulfate conjugation, however, is well developed and becomes the major route of acetaminophen conjugation in these children as shown below.

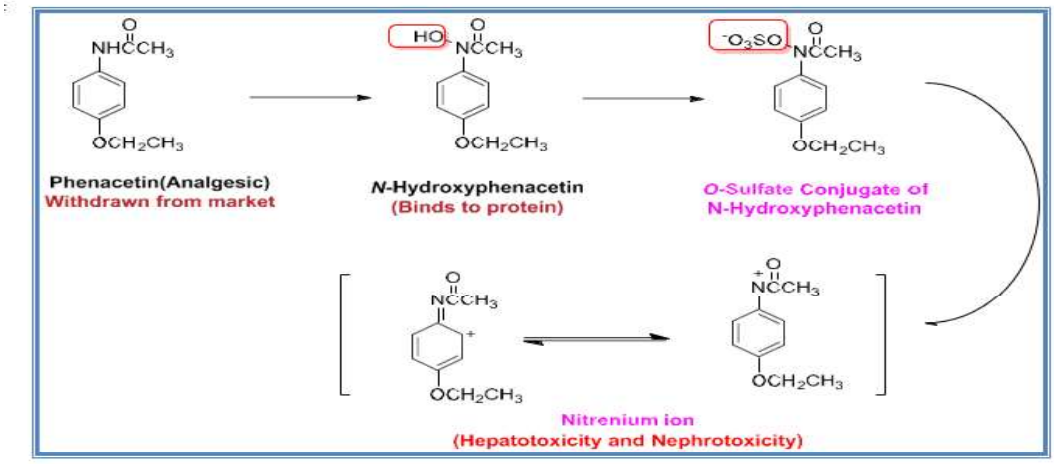


Main metabolites in adult

Main metabolites in infants

Although sulfate-conjugation is a detoxification reaction, it may produce reactive toxic molecule if it occurs on some N-OH containing compounds.

The hepatotoxicity and nephrotoxicity associated with phenacetin is due to its metabolism to *N*-hydroxyphenacetin and subsequently conjugated with sulfate. The *O*-sulfate conjugate of *N*-hydroxyphenacetin generates chemically reactive electrophile (nitrenium ion) binds covalently to proteins as shown below.

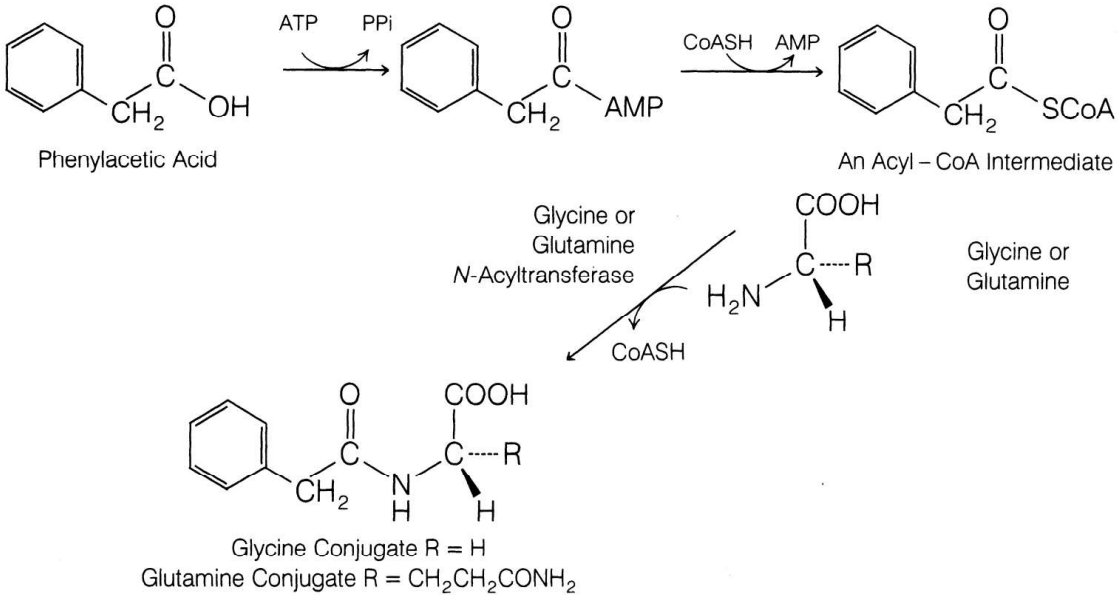


This pathway and arene oxides pathway represent metabolic pathways lead to reactive intermediates that are responsible toxicity associated with some drugs and xenobiotics.

C.Amino Acids Conjugation(Glycin&Glutamine)

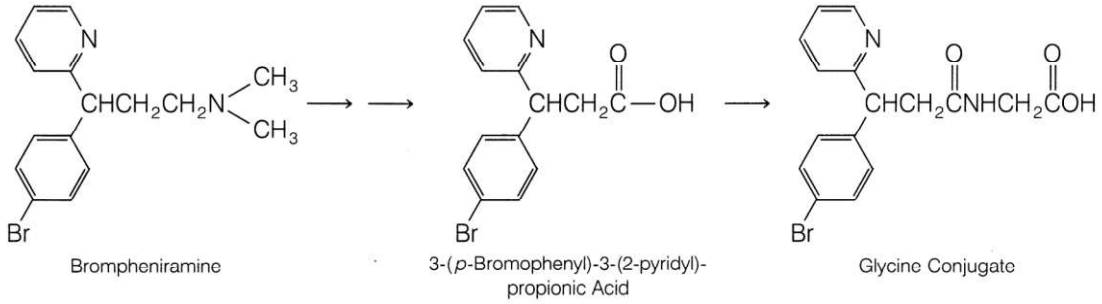
Amino acids conjugation is less frequent than glucuronide conjugation (glucuronidation). The functional group undergoing amino acids conjugation is carboxyl group (COOH) particularly aromatic acids and arylalkyl acids.

In contrast with glucuronic acid and sulfate, glycine and glutamine are not converted to activated coenzymes, instead, the carboxylic acid (substrate) is activated with adenosine triphosphate (ATP) and coenzyme A (CoA) to form an acyl-CoA complex. This complex (intermediate), in turn, acylates glycine or glutamine by the aid of glycine or glutamine *N*-acyltransferase enzymes.

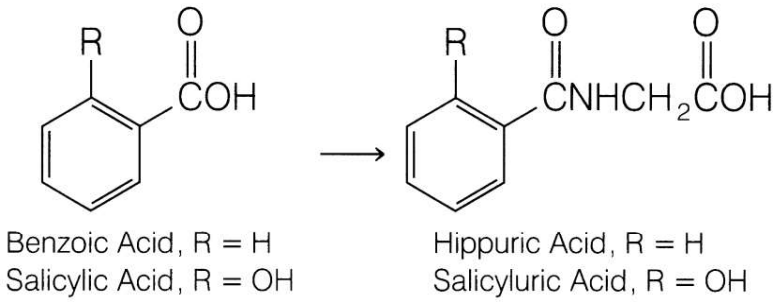


Formation of glycine and glutamine conjugates of phenylacetic acid

For example, brompheniramine is oxidized to a propionic acid metabolite that is conjugated with glycine as follows:



Benzoic acid and salicylic acid undergo glycine conjugate as shown below:



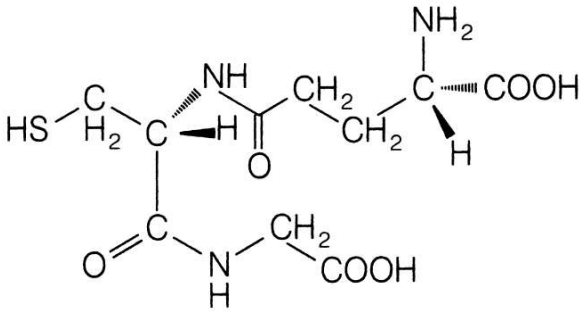
D. Glutathione (GSH) or Mercapturic Acid Conjugates (Conjugation)

GSH conjugation is an important pathway for detoxifying chemically reactive electrophilic compounds.

Reactive electrophilic species exert their toxicity (e.g., tissue necrosis, carcinogenicity, mutagenicity, teratogenicity) by combining covalently with nucleophilic groups present in vital cellular proteins and nucleic acids.

GSH protects vital cellular constituents against chemically reactive species by its nucleophilic SH group. The SH group reacts with electron-deficient compounds to form *S*-substituted GSH adducts

GSH is a tripeptide (γ-glutamyl-cysteinyl glycine, structure below.



Glutathione

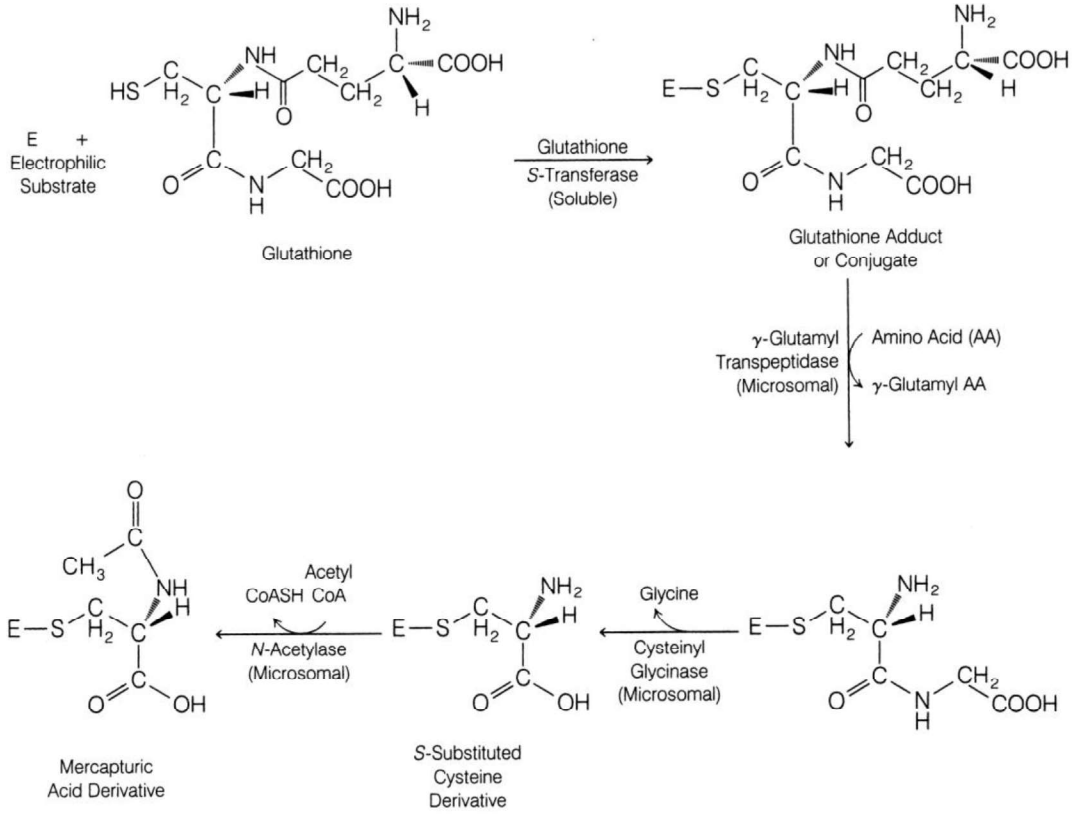
Xenobiotics conjugated with GSH usually are not excreted as such, but undergo further biotransformation to give *S*-substituted *N*-acetylcysteine products called mercapturic acids.

This process involves enzymatic cleavage of two amino acids (namely, glutamic acid and glycine) from the initially formed GSH adduct and subsequent N-acetylation of the remaining *S*-substituted cysteine residue.

Conjugation of substrates with GSH is catalyzed by enzymes known as GSH *S*-transferases.

Unlike other conjugative phase II reactions, GSH conjugation does not require the initial formation of an activated coenzyme or substrate. The inherent reactivity of the nucleophilic GSH toward an electrophilic substrate usually provides sufficient driving force.

A major structural requirement for the substrates susceptible to GSH conjugation is to be sufficiently electrophilic.



Formation of GSH conjugates of electrophilic xenobiotics or metabolites (E) and their conversion to mercapturic acids

As discussed previously, arene oxides and epoxides are intermediary products formed from CYP oxidation of aromatic compounds (arenes) and olefins, respectively are "neutralized" or detoxified by GSH *S*-conjugation.

2. Acetylation

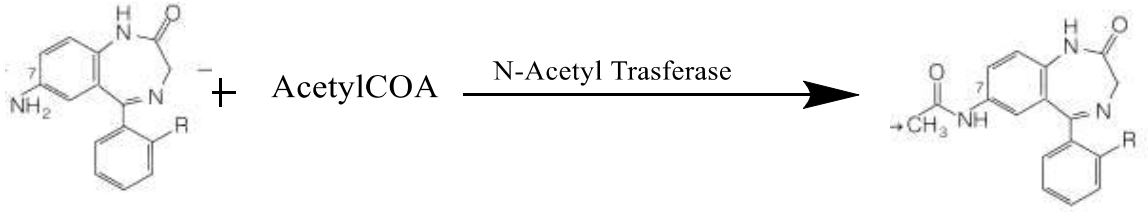
The objective of this metabolic pathway is to terminate the biological activity and detoxification of drugs and xenobiotics.

The functional group undergoing acetylation is primary amino group (NH_2), this includes:

a. Primary aromatic amines ($ArNH_2$),
b. Sulfonamides ($H_2NC_6H_4SO_2NHR$).

c. Hydrazines (-$NHNH_2$).
d. Hydrazides (-$CONHNH_2$).
e. Primary aliphatic amines (RNH_2; $PhNH_2$).

The amide derivatives formed from acetylation of these amino functionalities are generally inactive and nontoxic. A few reports indicate, that acetylated metabolites may be as active as parent compounds (e.g., *N*-acetylprocainamide), or more toxic than parent compounds (e.g., *N*-acetylisoniazid). Water solubility is not enhanced greatly by *N*-acetylation. The activated coenzyme is Acetyl-CoA, and the catalytic enzymes are N-acetyltransferases as shown below.



The acetylation pattern of several drugs (e.g., isoniazid, hydralazine, procainamide) in the human population displays a bimodal character in which the drug is conjugated either rapidly or slowly with acetyl-CoA. This phenomenon is termed acetylation polymorphism. This variation is genetic and is caused mainly by differences in *N*-acetyltransferase activity.

Individuals are classified as either:

a. Slow acetylator (e.g., Egyptians and some Western European groups)
b. Rapid acetylator (e.g., Eskimos and Asians)
c. Other populations are intermediate between these two extremes.

Because of the bimodal distribution of the human population into rapid and slow acetylators, there is a significant individual variation in therapeutic and toxicological responses to drugs displaying acetylation polymorphism.

Slow acetylators seem more likely to develop adverse reactions, whereas rapid acetylators are more likely to show an inadequate therapeutic response to standard drug doses.

The antituberculosis drug isoniazid illustrates an example on acetylation polymorphism.

3. Methylation

The objective of this metabolic pathway is to:

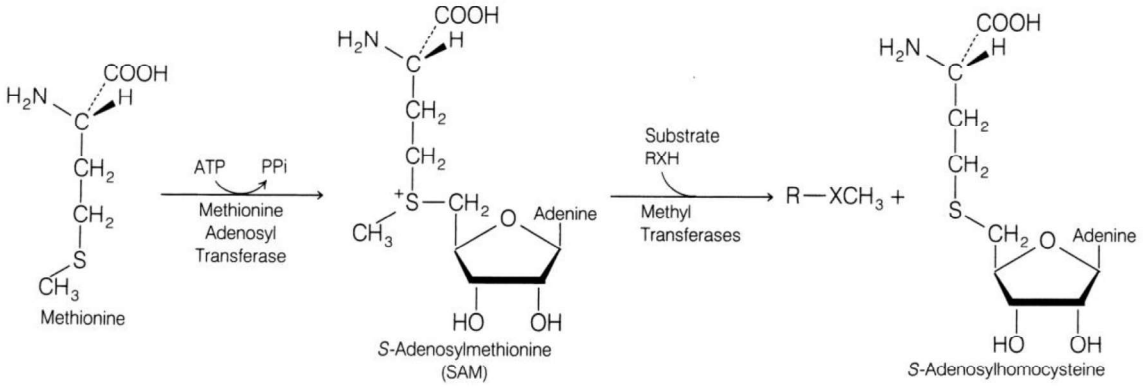
a) Terminate the biological activity of biogenic amines (e.g., norepinephrine , dopamine, serotonin, and histamine).
b) The biosynthesis of many endogenous compounds (e.g., epinephrine and melatonin).

Methylation, represents only a minor pathway for the metabolism of drugs and xenobiotics.

Methylation generally does not lead to polar or water-soluble metabolites, except when it creates a quaternary ammonium derivative.

Most methylated products tend to be pharmacologically inactive, although there are a few exceptions.

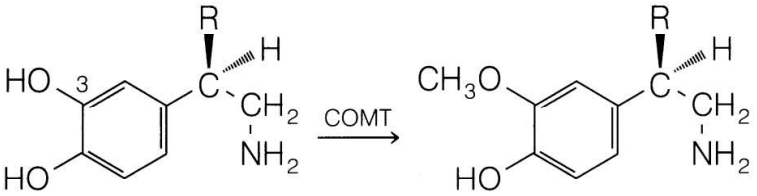
The activated coenzyme involved in methylation reactions is *S*-adenosylmethionine (SAM). Methylation is catalyzed by various Methyltransferases (MTs) as shown below:



MTs importances in the metabolism of xenobiotics include:

catechol-*O*-methyltransferase (COMT), phenol-*O*-methyltransferase, nonspecific *N*-methyltransferases and *S*-methyltransferases.

The enzymes, COMT carries out *O*-methylation of important neurotransmitters as norepinephrine and dopamine and thus terminates their activity.

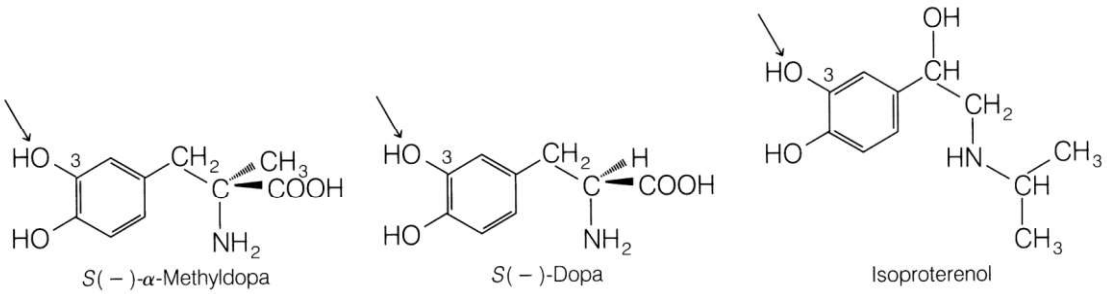


Norepinephrine, R = OH
Dopamine, R = H

Normetanephrine, R = OH
3-Methoxytyramine, R = H

Transferases that specifically methylate histamine, serotonin, and epinephrine are not usually involved in the metabolism of xenobiotics**.**

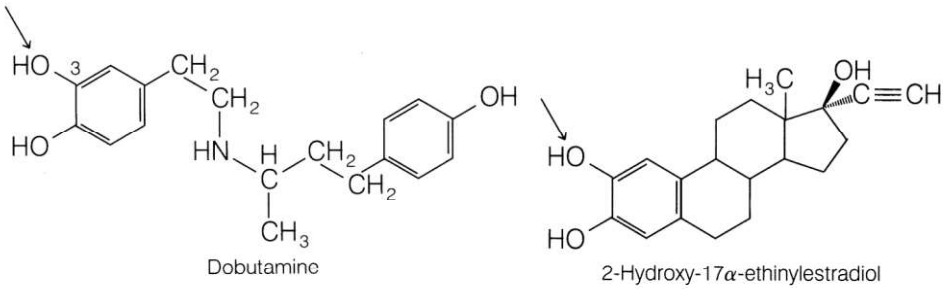
Examples of drugs that undergo significant *O*-methylation by COMT are illustrated below:



S(−)-α-Methyldopa

S(−)-Dopa

Isoproterenol

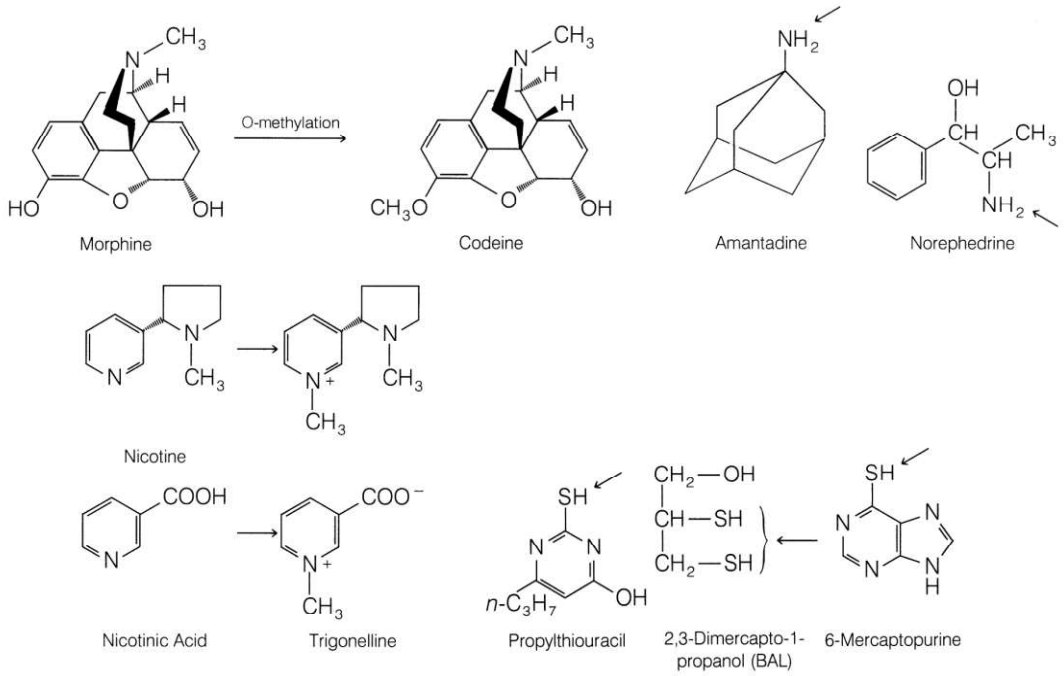


Dobutamine

2-Hydroxy-17α-ethinylestradiol

In the above four drugs, COMT selectively *O*-methylates only the phenolic OH at C-3. Bismethylation does not occur. COMT needs catecholic functionality to carry out methylation as above.

Other examples of drugs that undergo significant *O*-methylation, N- methylation & S-methylation are illustrated below:



Factors Affecting Drug Metabolism

Drugs and xenobiotics often are metabolized by several different phase I and phase II pathways to give several metabolites.

The relative amount of any particular metabolite is determined by the concentration and activity of the enzyme(s) responsible for the biotransformation.

The rate of metabolism of a drug is particularly important for its pharmacological action as well as its toxicity.

If the rate of metabolism of a drug is decreased, this generally, increases the intensity and duration of the drug action. In addition, decreased metabolic elimination may lead to accumulation of toxic levels of the drug and vis-versa.

An increased rate of metabolism decreases the intensity and duration of action as well as the drug's efficacy.

In addition to the concentration and activity of the enzyme(s), many other factors may affect drug metabolism. These are

1. **Chemical factor (Chemical Structure):**
2. **Biological factors:** Age, species and strain, genetic or hereditary factors, sex, enzyme induction, enzyme inhibition, physiological or disease state, drug dosing, nutritional status and drug route of administration.

Stereochemical Aspects of Drug Metabolism

The preferential interaction of one stereoisomer with drug-metabolizing enzymes may lead differences in metabolic pathways and the amount of metabolites for the two enantiomers

This is due to many factors which include:

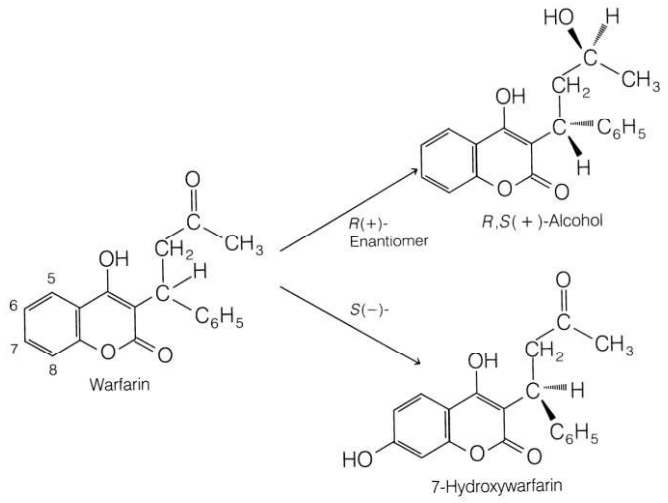
a. **Substrate stereoselectivity.**
b. **Product stereoselectivity.**
c. **Regioselectivity**

a. Substrate stereoselectivity

The term ***substrate stereoselectivity*** is used to denote a preference for one stereoisomer as a substrate for a metabolizing enzyme or metabolic process.

For instance, the (+) enantiomer of propranolol undergoes more rapid metabolism than the corresponding (-) enantiomer.

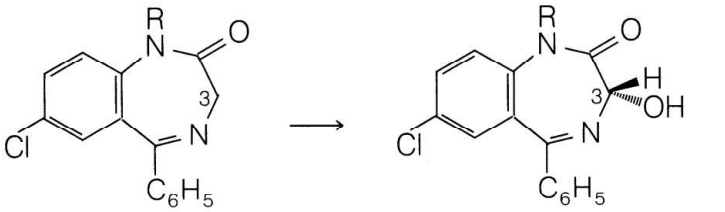
Dramatic differences in the metabolic profile of two enantiomers of warfarin also have been noted. The (*S*) (-)-isomer is 7-hydroxylated (aromatic hydroxylation), whereas the (*R*)(+)-isomer undergoes keto reduction to yield the (*R*,*S*) warfarin alcohol as the major plasma metabolite as shown below:



b. product stereoselectivity.

It means the preferential metabolic formation of a stereoisomeric product (formation of one stereoisomer as predominant metabolic product).

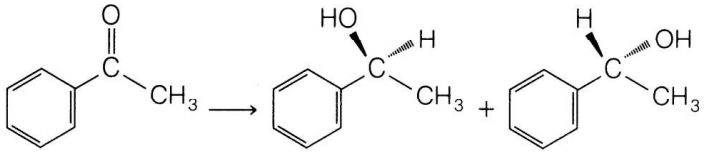
Oxidative biotransformations display product stereoselectivity as shown below:



Diazepam, R = CH_3
Desmethyldiazepam, R = H

(3*S*) *N*-Methyloxazepam, R = CH_3
S(+)-Oxazepam, R = H

The, bio reduction of ketone xenobiotics, as a general rule, produces predominantly one stereoisomeric (*S*) (-)- alcohol as shown below:



Acetophenone

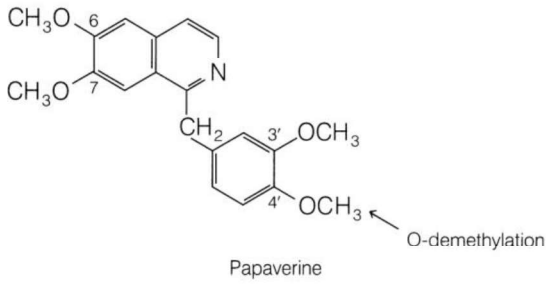
S(−)-Methyl Phenyl Carbinol (75%)

R(+)-Methyl Phenyl Carbinol (25%)

c. Regioselectivity

The term **regioselectivity** describes the selective metabolism of two or more similar functional groups (e.g., OCH3, OH, NO2) or two or more similar atoms that are positioned in different regions of a molecule.

For example, of the four methoxy groups present in papaverine, the 4-OCH3 group is regioselectively O-demethylated alcohol as shown below:



Papaverine

Pharmacologically Active Metabolites

The traditional idea that drugs metabolites are inactive and insignificant in drug therapy has changed dramatically in recent years. Increasing evidence indicates that many drugs are biotransformed to pharmacologically active metabolites that contribute to the therapeutic as well as toxic effects of the parent compounds. Prodrugs (pharmacologically inactive) are bioactivated enzymatically to the pharmacologically active drug as in chloramphenicol palmitate which is ester prodrug hydrolyzed by esterase to the active drug (chloramphenicol).