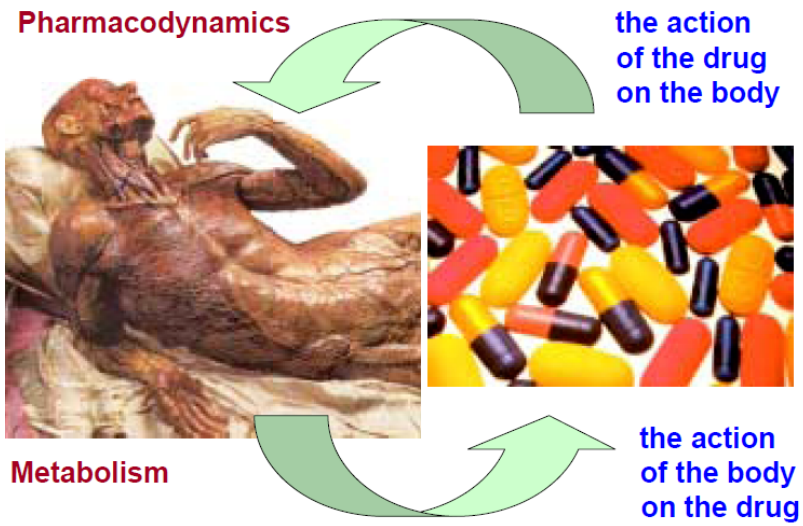
NEPHAR 305
Pharmaceutical Chemistry I



Drug Metabolism: Phase I

Assist.Prof.Dr. Banu Keşanlı

Drug Metabolism

- Drug's biochemical modification or degradation, usually through specialized enzymatic systems
- **Xenobiotic:** a chemical which is found in an organism but which is not normally produced or expected to be present in it
- Drug metabolism often converts lipophilic chemical compounds into more readily excreted polar products
- Duration and intensity of the pharmacological action of drugs is important
- Drug metabolism can result in toxication if the metabolite of a compound is more toxic than the parent drug or chemical
- or detoxication (process of preventing toxic entities from entering the body in the first place) by the activation or deactivation of the chemical

A **prodrug** is a pharmacological substance (drug) that is administered in an inactive (or significantly less active) form. Once administered, the prodrug is metabolised in vivo into an **active metabolite**.

Importance of Drug Metabolism

Basic premise:

Lipophilic Drugs ⇒ Hydrophilic Metabolites
(Not excreted) (Excreted)

Water soluble ⇒ increased renal excretion
and
Decreased tubular re-absorption of lipophilics

Importance of Drug Metabolism

Metbolism → Termination of Drug
- Bioinactivation
- Detoxification
- Elimination

Metabolism → Bioactivation
- Active Metabolites
- Prodrugs
- Toxification

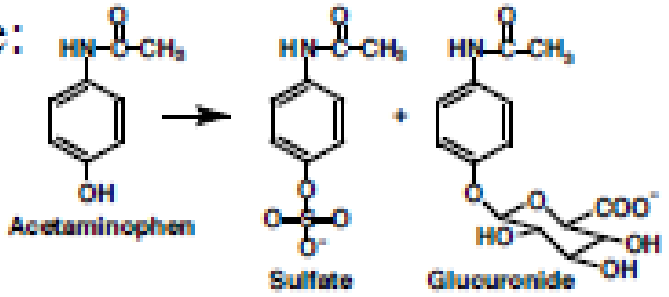
Phase-1 Metabolism Description

- Phase 1 = "Functionalization" Reactions
  - New polar functional groups.
  - Interchange existing functional groups
  - Unmask existing polar groups.

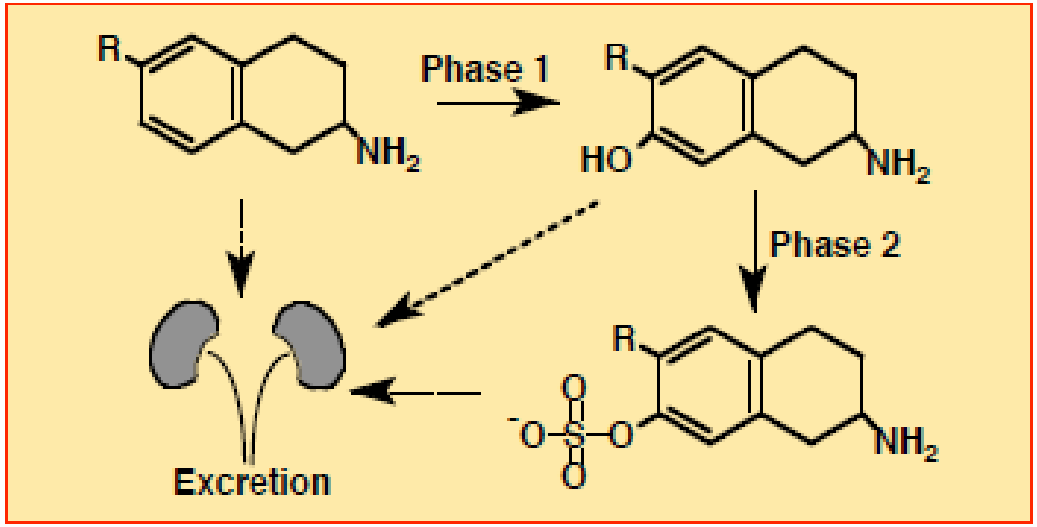
  - enhance excretion
    RH --> ROH (more water soluble)
  - prepare for phase 2
    RH --> ROH (functional handle)

Phase-2 Metabolism Description

- Phase 2 = "Conjugation" Reactions
  - Acts on parent drug or
  - Acts on phase 1 metabolite.
- Links to endogenous, polar, ionizable cpd.
- Purpose: enhance excretion.
- Reaction types include:
  - Glucuronidation
  - Sulfate formation



Phase-1 & Phase-2 Complimentary
NOT Mutually Exclusive



Phase I or Functionalization Reactions

Oxidative Reactions

- Oxidation of aromatic moieties
- Oxidation of olefins
- Oxidation at benzylic, allylic carbon, carbon atoms α to carbonyl and imines
- Oxidation at aliphatic and alicyclic carbon
- Oxidation involving carbon-heteroatom systems:

  Carbon-nitrogen systems (aliphatic and aromatic amines; N-dealkylation, oxidative deamination, N-oxide formation, N-hydroxylation)

  Carbon-oxygen systems (O-dealkylation)

  Carbon-sulfur systems (S-dealkylation, S-oxidation, and desulfuration)
- Oxidation of alcohols and aldehydes
- Other miscellaneous oxidative reactions

Reductive Reactions

- Reduction of aldehydes and ketones
- Reduction of nitro and azo compounds
- Miscellaneous reductive reactions

Hydrolytic Reactions

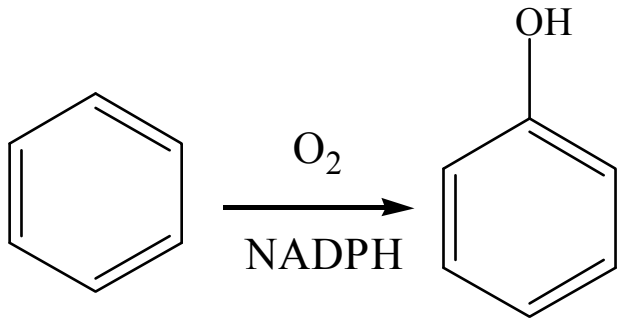
- Hydrolysis of esters and amides
- Hydration of epoxides and arene oxides by epoxide hydrase

Phase II or Conjugation Reactions

- Glucuronic acid conjugation
- Sulfate conjugation
- Conjugation with glycine, glutamine, and other amino acids
- Glutathione or mercapturic acid conjugation
- Acetylation
- Methylation

Transformation of Xenobiotics by Biological Systems

- **Phase I** and **Phase II** reactions are biotransformations of chemicals that occur during drug metabolism
- Oxidative biotransformations require both molecular oxygen and the reducing agent NADPH (reduced form of nicotinamide adenine dinucleotide phosphate)
- One atom of molecular oxygen (O_2) is introduced into the substrate R-H to form R-OH and the other oxygen atom is incorporated into H_2O

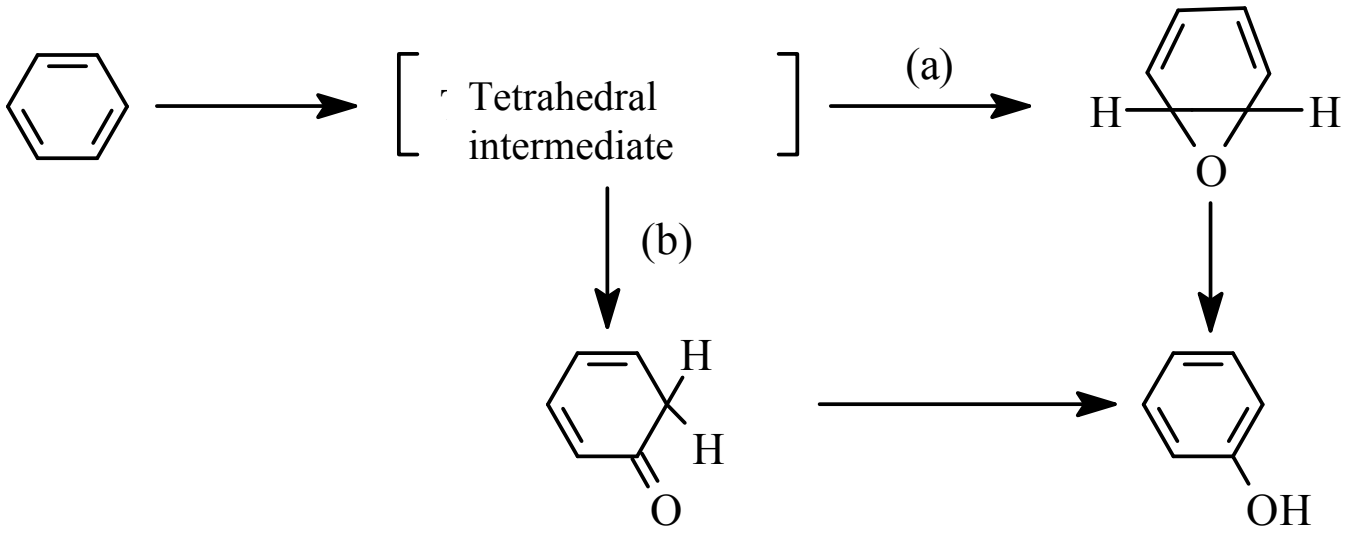


parent compound $\xrightarrow{\text{biotransformation}}$ metabolite

lipophilic agent $\xrightarrow{\text{biotransformation}}$ hydrophilic product

Aromatic Hydroxylation

- Major route of metabolism for many drugs containing a phenyl group (and aromatic)
- Proceeds initially with an "arene oxide" intermediate
- Hydroxylation occurs at para position
  - Activated rings – electron donating substituents
  - Deactivated rings don't get oxidized – toxic
- Dihydrol metabolite formation is possible
- Undergoes further conversions to polar water soluble glucuronide or sulfate conjugates, which are readily excreted in the urine



Oxidation Involving Carbon-Heteroatom Systems

- Hydroxylation of α-carbon atom attached to the heteroatom (N, O, S) results in unstable intermediate which decomposes via cleavage of carbon – heteroatom bond

- Hydroxylation or oxidation of heteroatom (N, O, S) could fall under these mechanisms: N-hydoxylation, N-oxide formation, sulfoxide, sulfone formation

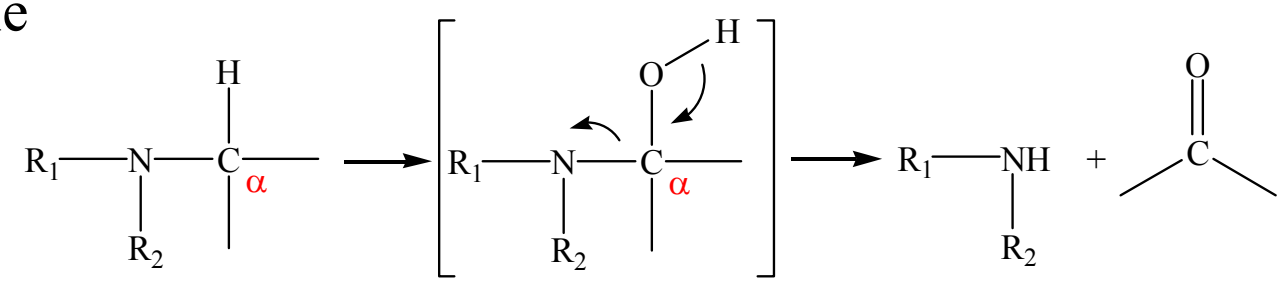
- Structural factors determine the metabolic pathway – complicated

- Nitrogen functionalities such as amines, amides are found in natural products (morphine, nicotine etc) and in numerous drugs (antihistamines, phenothiazine etc)

Tertiary aliphatic and Alicyclic amines

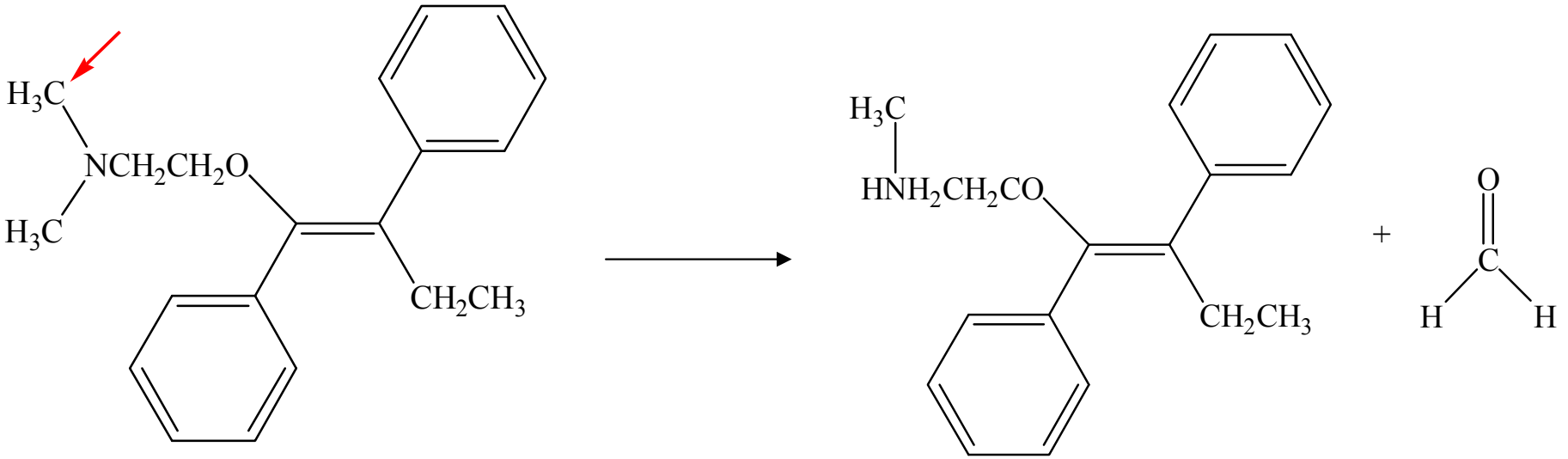
Oxidative N-dealkylation: Oxidative removal of alkyl groups

Small groups such as methyl, ethyl, isopropyl removed rapidly, t-butyl is impossible



Tertiary amine | Carbinolamine | Secondary amine | Carbonyl (aldehyde or ketone)

Antiestrogenic agent Tamoxifen (Nolvadex)



Tamoxifen

Secondary and Primary Amines

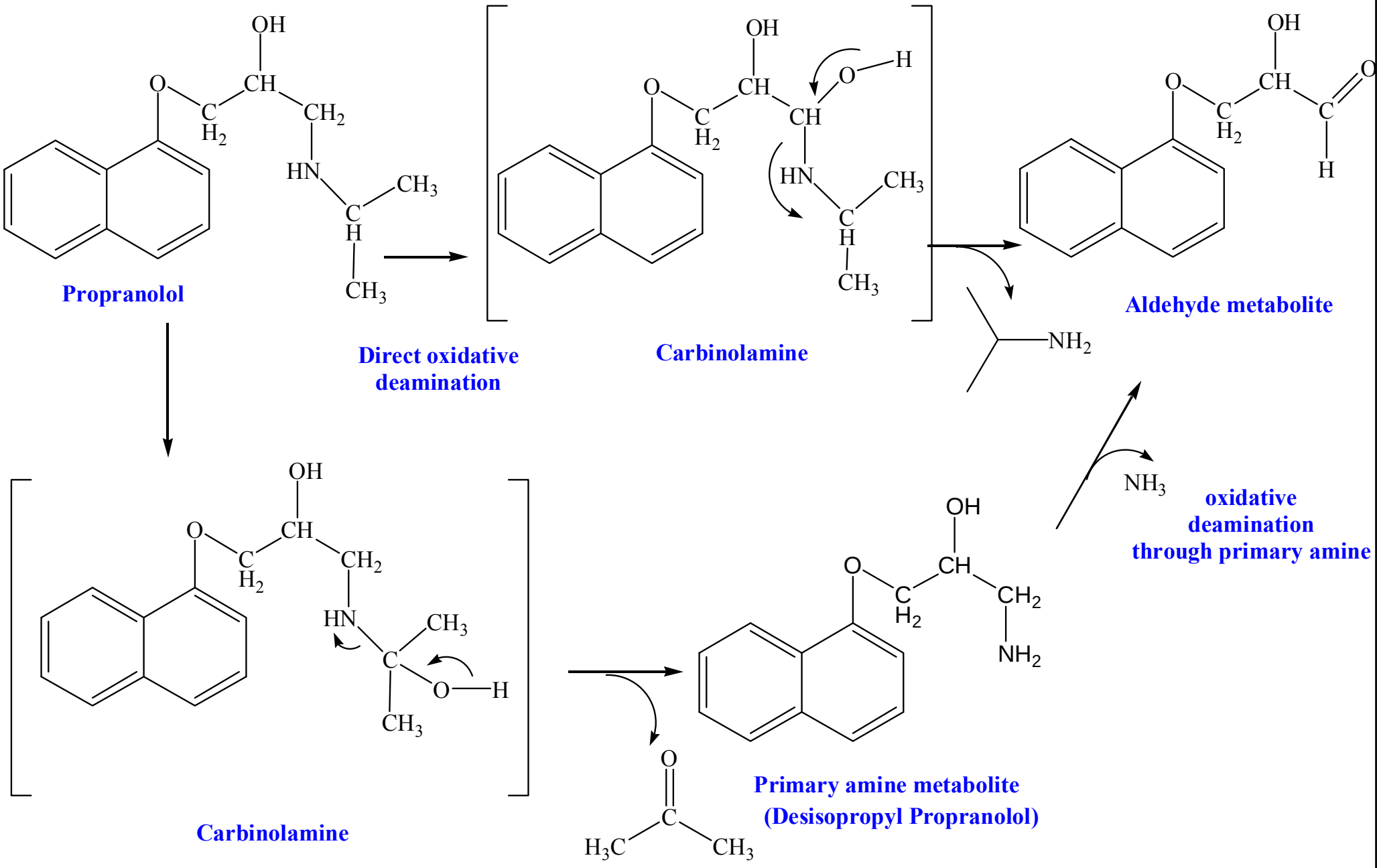
- Oxidative N-dealkylation
- Oxidative deamination
- N-oxidation

✓ Carbinolamine pathway to give corresponding primary amine metabolite through N-dealkylation

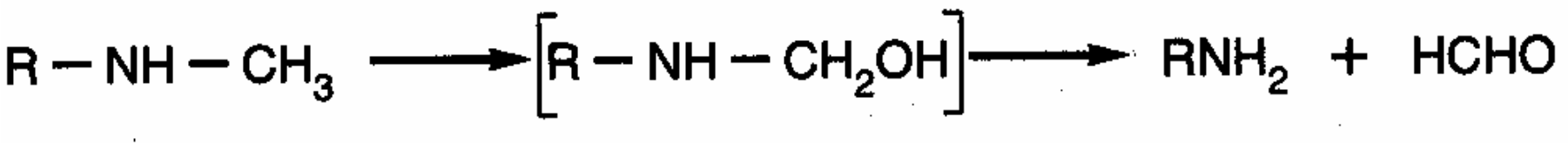
✓ Which then is susceptible to oxidative deamination

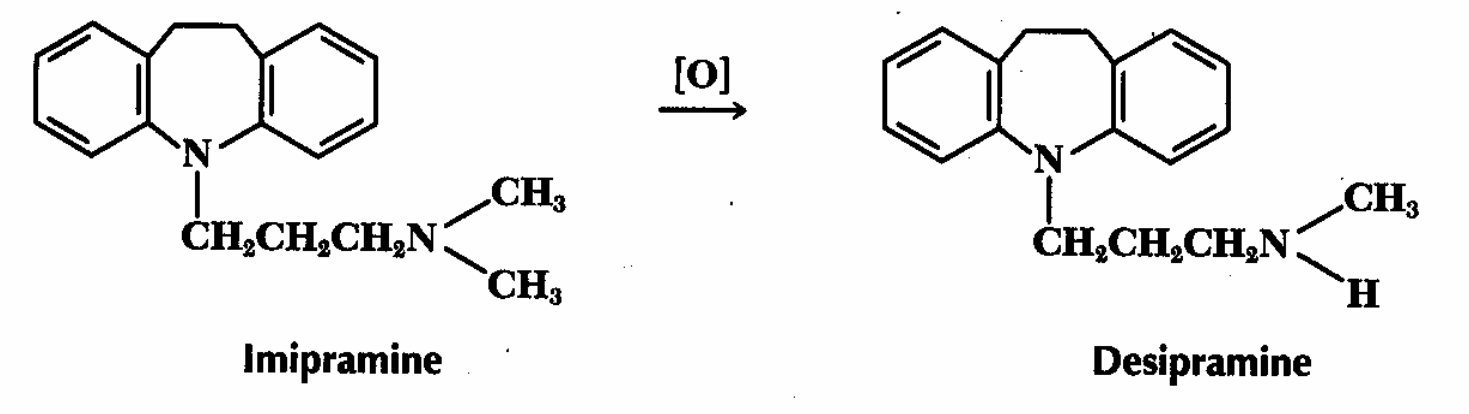
- initial α-C hydroxylation followed by C-N bond cleavage to give carbonyl metabolite and ammonia

➢ Metabolism of Propranolol both by direct deamination and deamination of its primary amine metabolite

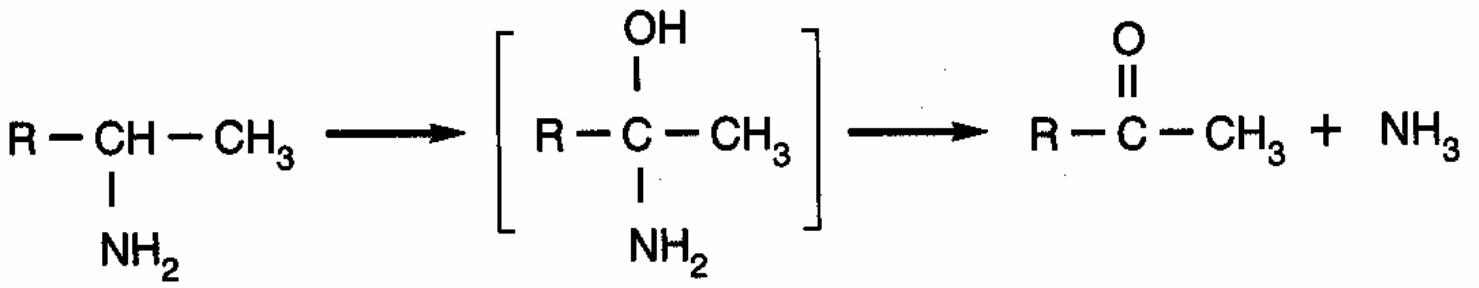


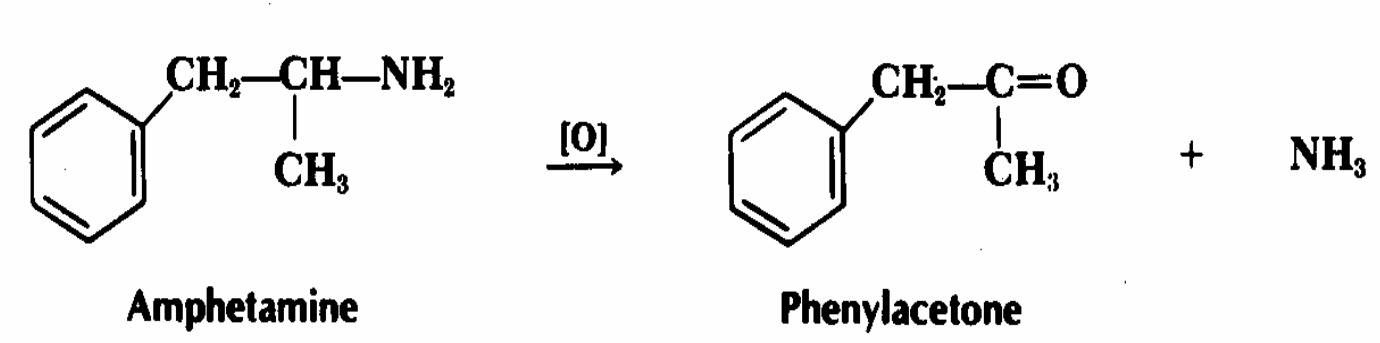
N-Dealkylation



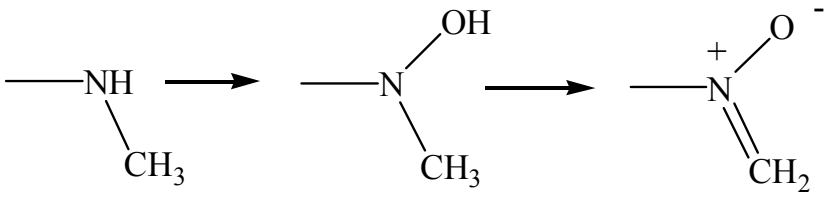


Oxidative Deamination

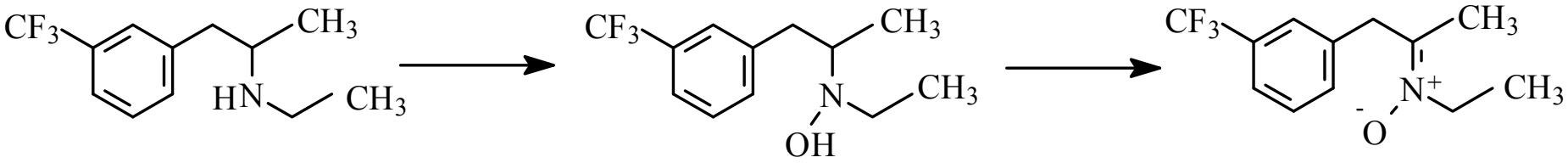




➢ Metabolic N-oxidation of secondary amine leads to N-oxygenated products, N-hydroxylamines which are susceptible to further oxidation giving nitrone metabolites



Secondary amine — Hydroxylamine — Nitrone



Hydroxylation — Nitrone

➢ Secondary amines undergo oxidative dealkylation and deamination more than N-oxidation

➢ Primary aliphatic amines are biotransformed by oxidative deamination or by N-oxidation

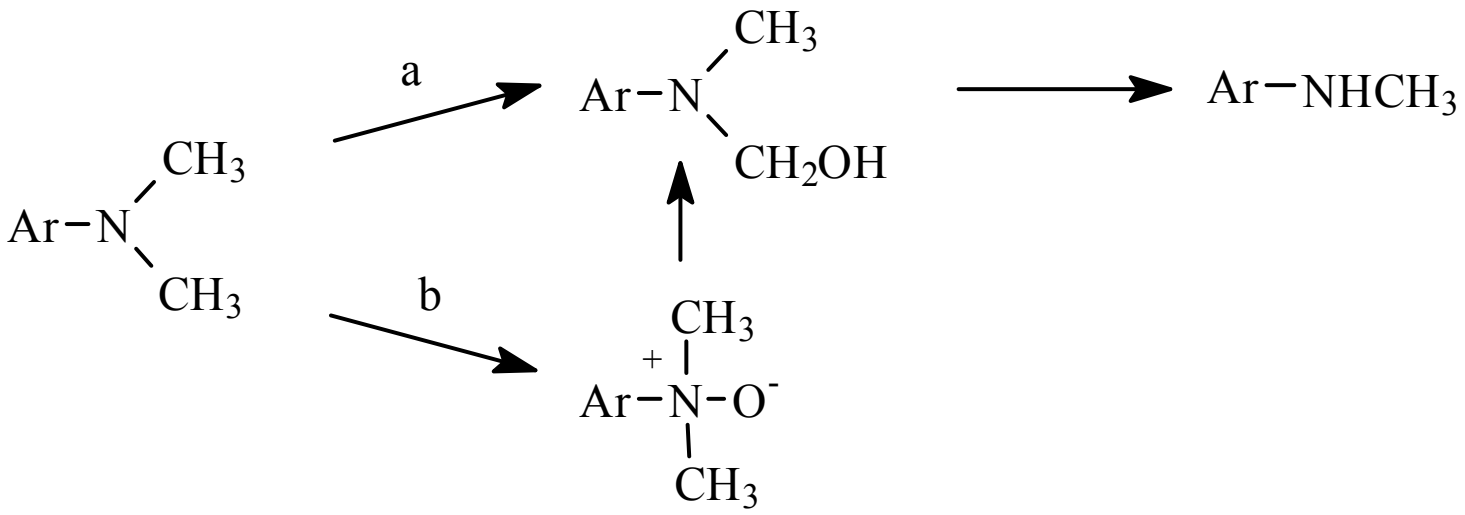
➢ Monoamine oxidase (MAO) enzymes are responsible for oxidative deamination

➢ Structural features, e.g. α-substituents determine whether C or N oxidation will occur

• If no hydrogen atom on α-C then α-C hydroxylation is impossible

Aromatic Amines and Heterocyclic Nitrogen Compounds

- Tertiary aromatic amines undergo oxidative N-dealkylation and N-oxide formation
- Secondary aromatic amines undergo N-dealkylation and N-oxide formation
- Primary amines undergo N-oxidation generating N-hydroxylamine metabolite
- Oxidation of hydroxylamine derivative to nitroso derivative is possible
- N-oxidation is minor compared to other biotransformations such as N-acetylation aromatic hydroxylation
- Conjugation pathways are observed (e.g. sulfate conjugation)



Oxidation of Alcohols and Aldehydes

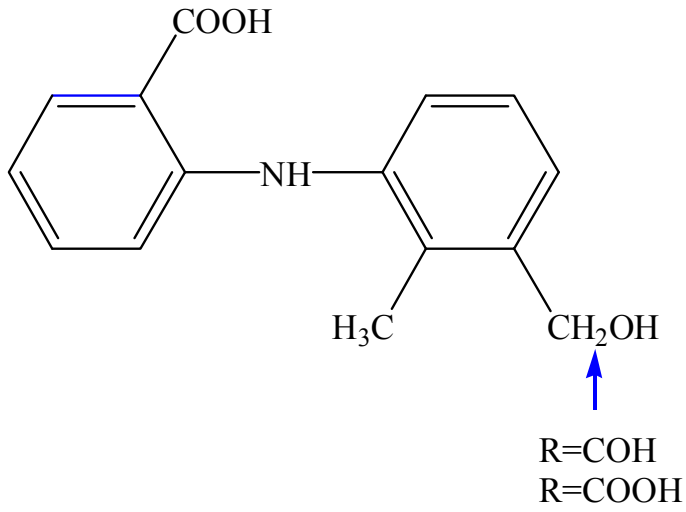
- Primary alcohols are oxidized to aldehydes which often undergo facile oxidation to generate polar carboxylic acid derivatives

- Secondary alcohols are oxidized to ketones – more likely to form conjugates or be reduced back to alcohol form

- Catalyzed by soluble alcohol dehydrogenase present in liver and other tissues

$$RCH_2OH + NAD^+ \rightleftharpoons RCHO + NADH + H^+$$

$$RCHO + NAD^+ \rightleftharpoons RCOOH + NADH$$



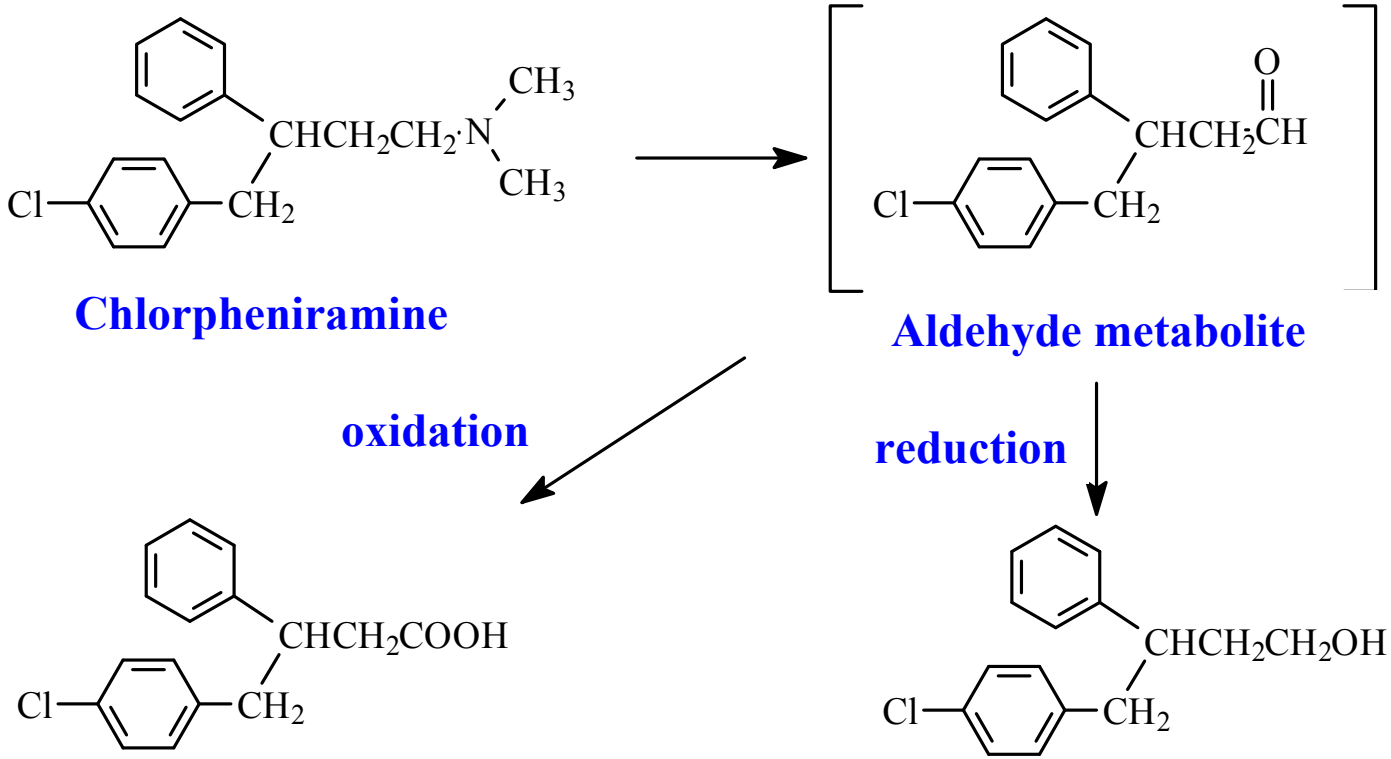
Mefenamic acid

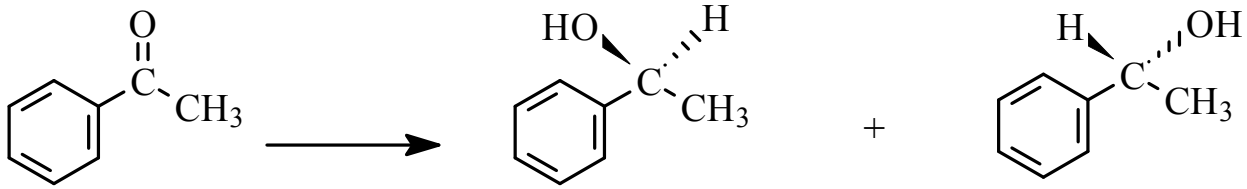
Reductive Reactions

- Plays an important role in the metabolism of many compounds containing carbonyl, nitro and azo groups
- Bioreduction of carbonyl compounds generates alcohol derivatives
- Nitro and azo reductions lead to amino derivatives
- Hydroxyl and amino moieties of the metabolites are more susceptible to conjugation than the functional groups of the parent compounds
- Facilitate drug elimination

Reduction of Aldehydes and Ketones

- Carbonyl containing drugs and metabolites are common
- Aldehydes are more readily oxidized to carboxylic acids then reduced to alcohols
- Ketones are resistant to oxidation, mainly reduced to secondary alcohols
- *Aldo-keto reductase* enzymes are responsible for reduction
- Bioreduction of ketones often leads to the creation of asymmetric center thus 2 possible stereoisomeric alcohols

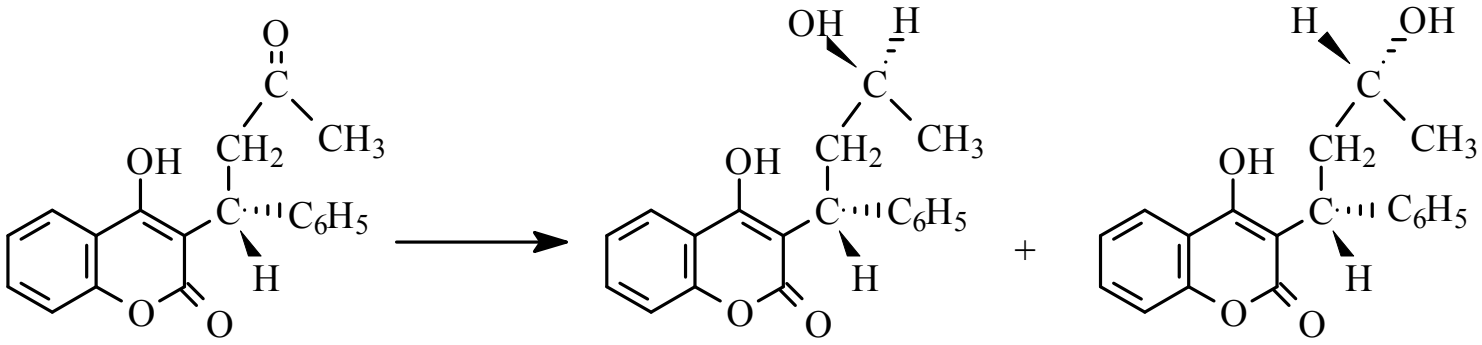

Chlorpheniramine
Aldehyde metabolite
oxidation
reduction



Acetophenone

S-(-)-Methyl
Phenyl Carbinol

R-(+)-Methyl
Phenyl Carbinol



R(+)-Warfarin

R,S-(+)-Alcohol
Major diastereomer

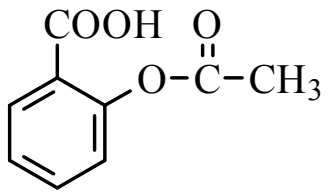
R,R-(+)-Alcohol
Minor diastereomer

Hydrolytic Reactions

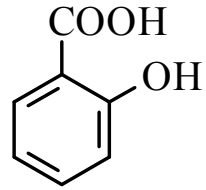
Hydrolysis of Esters and Amides

- Metabolism of ester and amide linkages in many drugs is catalyzed by hydrolytic enzymes in tissues and plasma
- Metabolic products formed (carboxylic acids, alcohols, phenols, amides) are polar
- Functionally more susceptible to conjugation and excretion than the parent ester or amide
- Hydrolysis is a major biotransformation pathway for ester functionality

- Many parent drugs have been chemically modified or derivatized to generate prodrugs to overcome some undesirable property (e.g. bitter taste, poor absorption, poor solubility, irritation at site of injection)
- Ester derivatives are ideal prodrug candidates
- Amides are hydrolyzed slower compared to esters



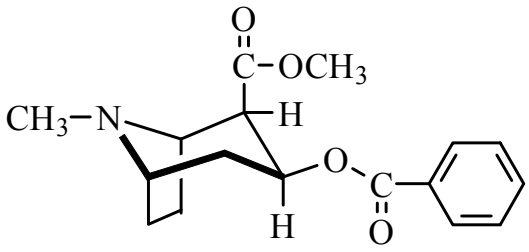
Aspirin
(Acetylsalicylic acid)



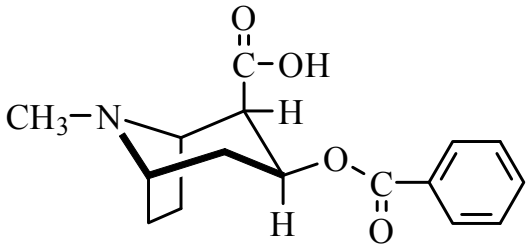
Salicylic acid

+ CH_3COOH

Acetic acid

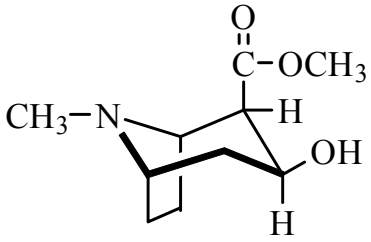


Cocaine

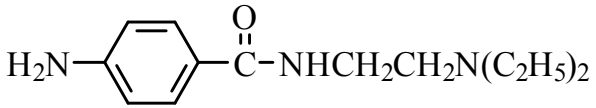


Benzoylecgonine

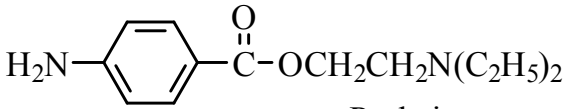
+



Methylecgonine



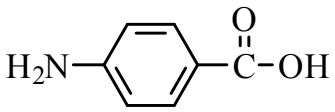
Procainamide

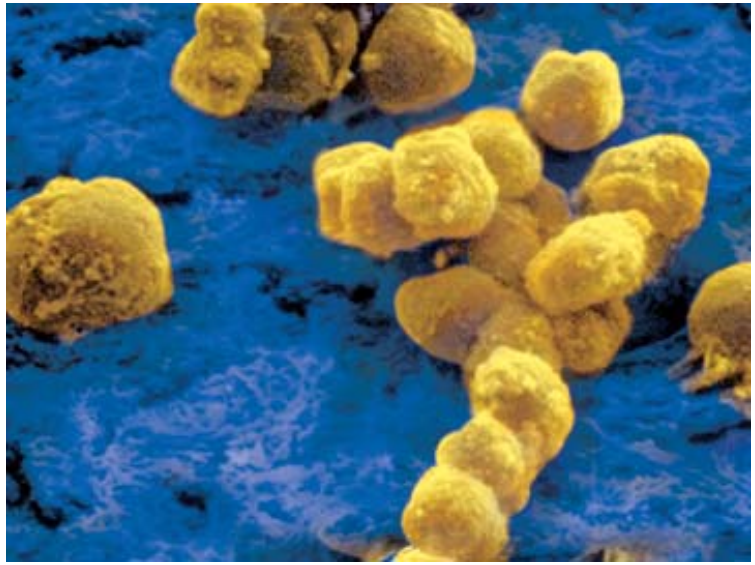


Procaine

Slow hydrolysis

Fast hydrolysis

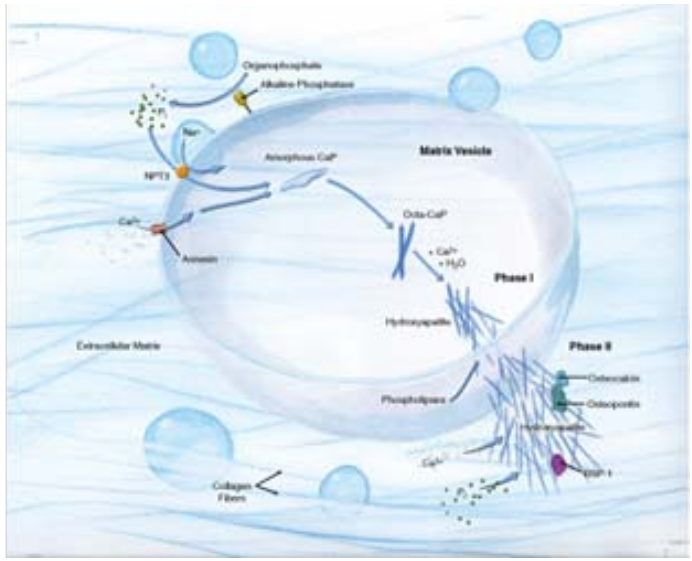




NEPHAR 305
Pharmaceutical Chemistry I

Drug Metabolism
Phase II

Prof. Dr. Hakkı Erdoğan
Assist.Prof. Banu Keşanlı

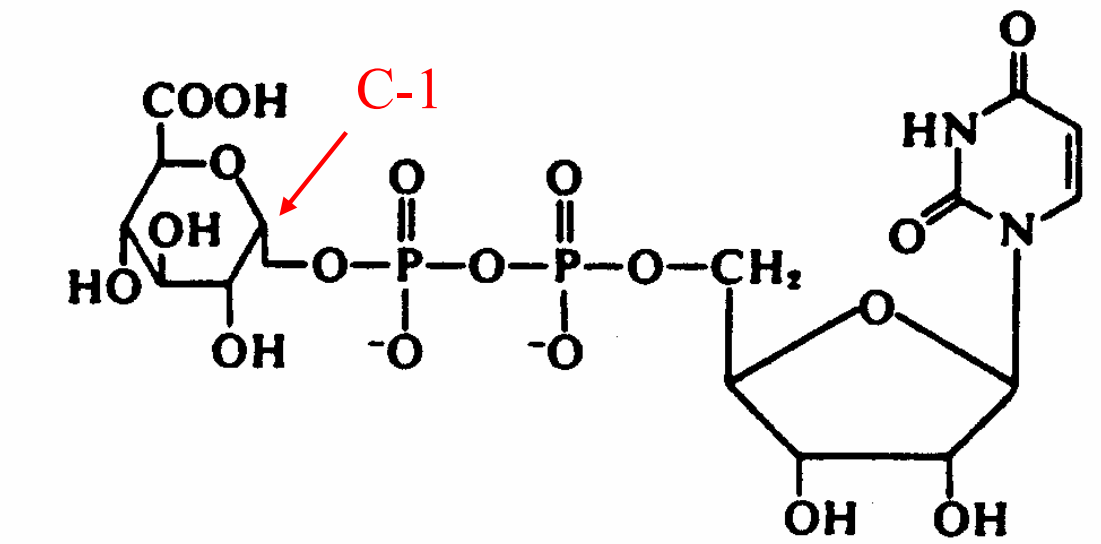


PHASE II REACTIONS

1. **Glucuronidation**
2. **Sulfate Conjugation**
3. **Acetylation**
4. **Amino Acid Conjugation**
5. **Methylation**
6. **Glutathione Conjugation**

Glucuronic Acid Conjugation

Uridine-5'-α-D-glucuronic Acid



Uridine-5'-diphospho-α-D-glucuronic acid (UDP-GA)

- The microsomal enzyme glucuronyl transferase conducts the donation of glucuronic acid from the endogenously synthesized UDPGA to various substrates to form glucuronide conjugates.
- Examples of such substrates are morphine and acetaminophen.

Glucuronic Acid Conjugation

- Most common conjugative pathway
- Greatly enhances water solubility
- Numerous functional groups can combine with it
- Readily available in body
- Has polar carboxyl and hydroxyl groups
- Products are called glucuronides

  RN-G; RO-G; RCOO-G; RS-G; RC-G glucuronides could form at C1 atom of β-glucuronide

- Phenolic and alcoholic hydroxyls are most common functional groups metabolized
- Glucuronidation is not fully developed in infants and children

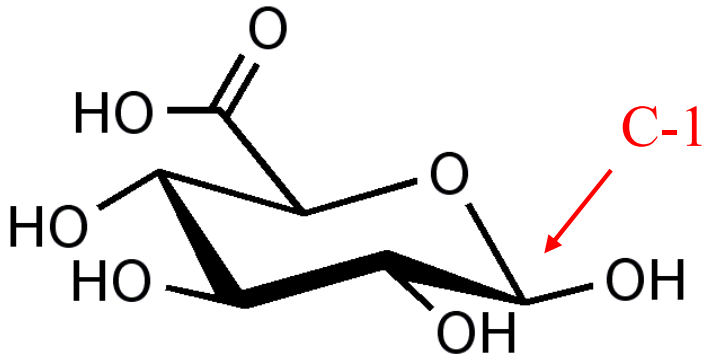
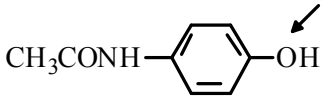
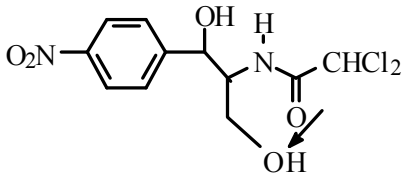
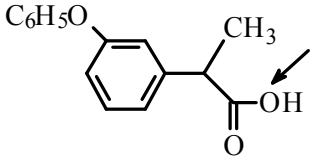
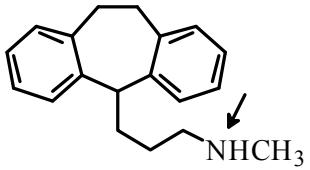
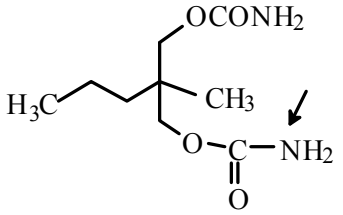
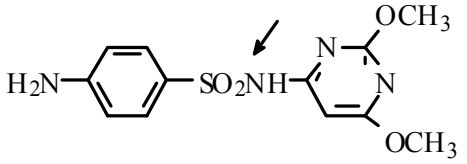


Table 5.10. Substrates forming Glucuronides

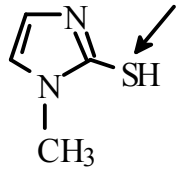
(R. B. Silverman "The Organic Chemistry of Drug Design and Drug Action" 1992, s.331)

Type	Compound	Formula
a) O-Glucuridation		
Hydroxyl (ether glucuronide) phenol	Acetaminophen	
Alcohol	Chloramphenicol	
Carboxyl (ester glucuronide)	Fenoprofen	
b) N- Glucuridation		
Amine	Desipramine	
Amide		
Carbamate	Meprobamate	
Sulfonamide	Sulfadimethoxine	

c) S- Glucuridation

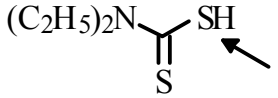
Sulfahydryl

Methimazole



Karboditiyonik asit

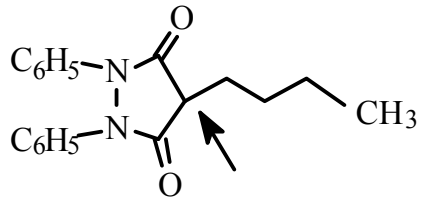
Disulfiram



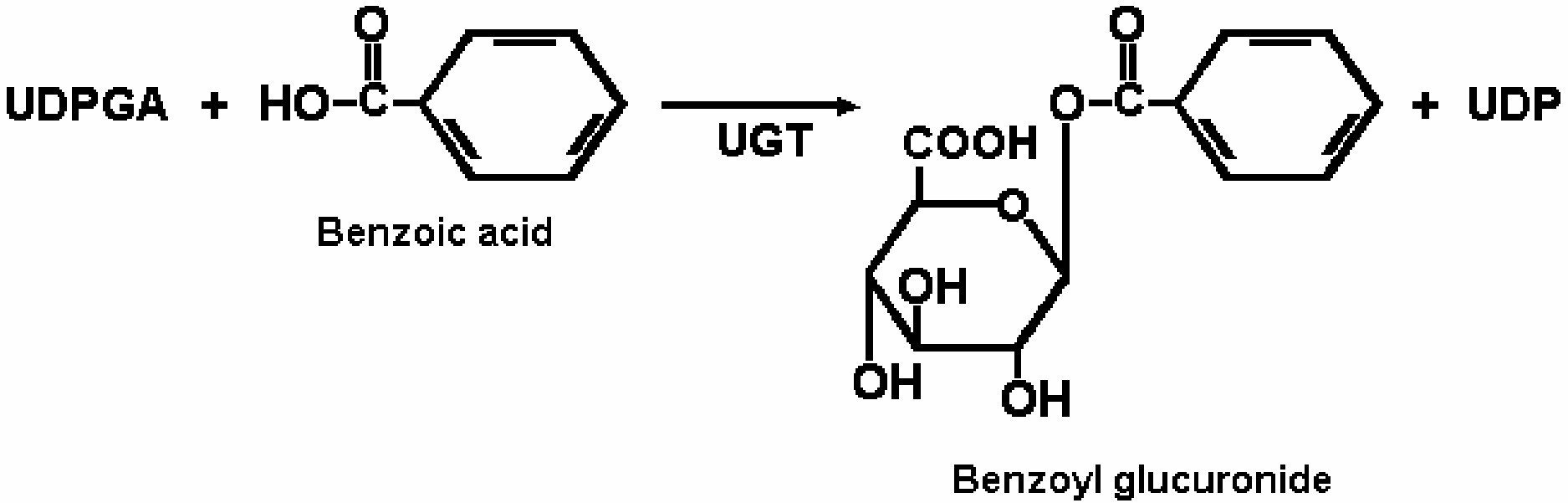
(reduced metabolite)

d) C- Glucuridation

Phenilbutazone

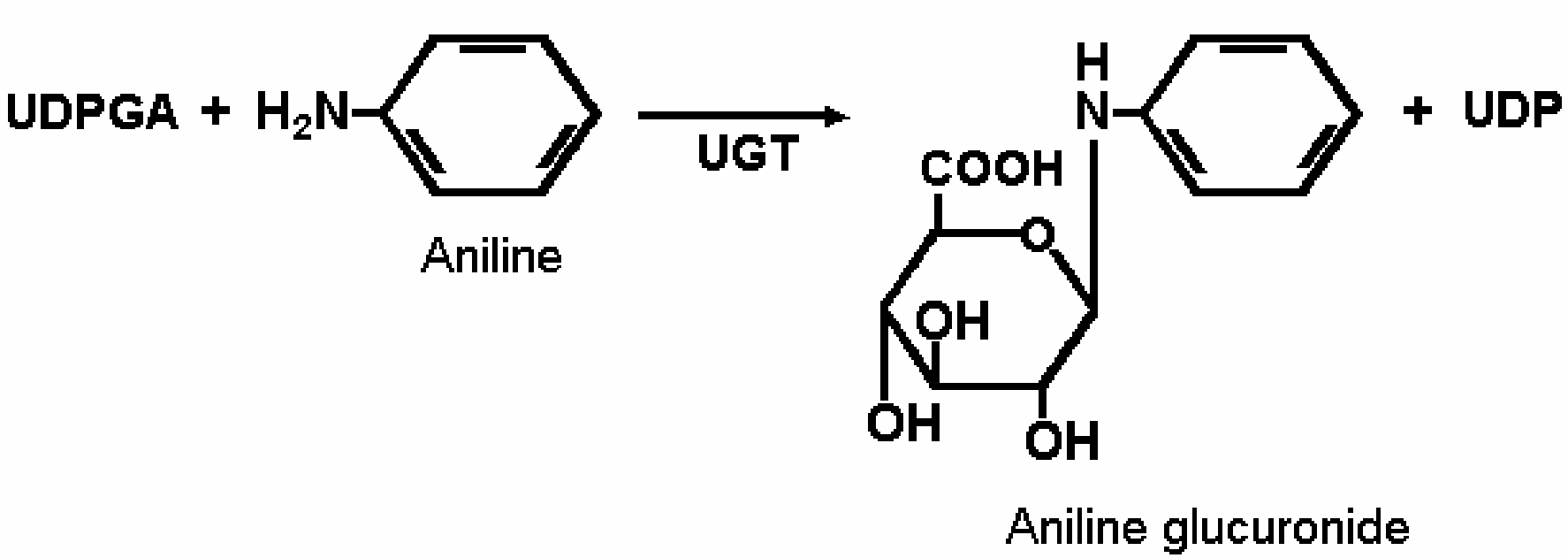


Glucuronidation of Benzoic Acid



UGT= UDP-α-D-Glucuronsyltransferase

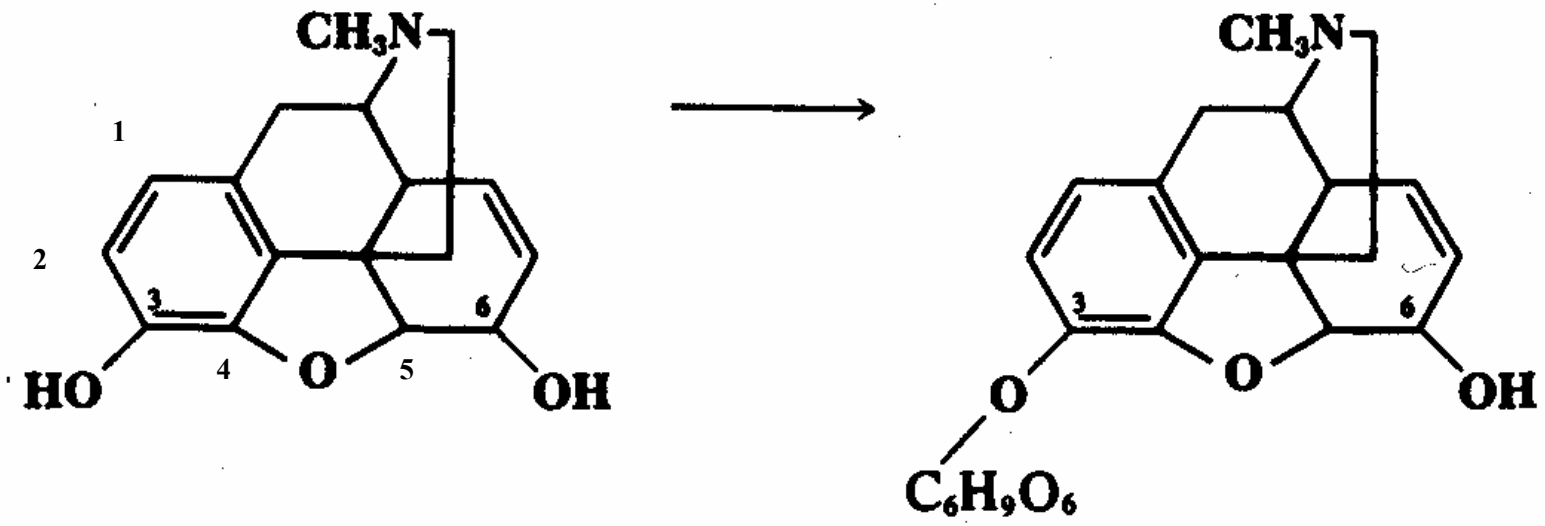
Glucuronidation of Aniline



Morphine Metabolism

Morphine → Morphine -6-glucuronide (active metabolite)

Morphine → Morphine -3-glucuronide (inactive metabolite)



Morphine

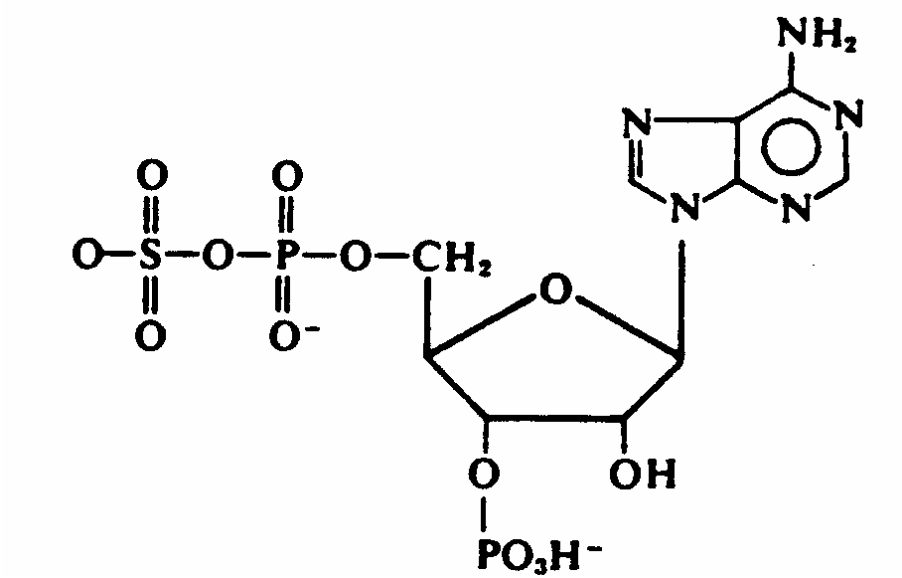
Morphine-3-glucuronide

Morphine -3-glucuronide is the major metabolite

A small amount of morphine undergoes N-demethylation

Sulfate Conjugation

3’-Phosphoadenosine-5’-phosphosulfate (PAPS)

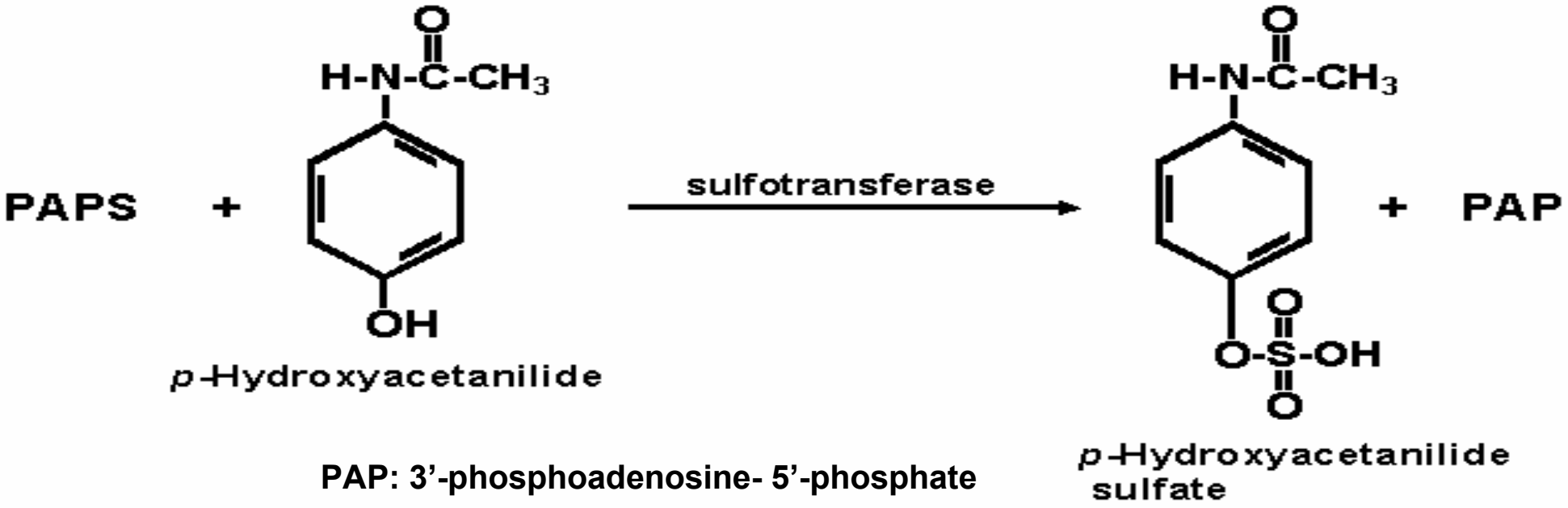


- The cytosolic enzyme sulfotransferase conducts the donation of sulfate from the endogenously synthesized PAPS to various substrates to form sulfate conjugates.
- An example of such substrate is acetaminophen.

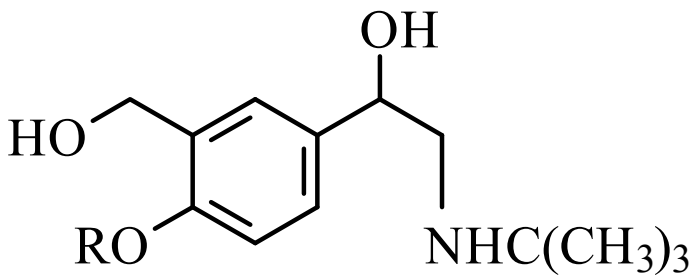
Sulfate Conjugation

- Occurs primarily with phenols and occasionally with alcohols, aromatic amines and N-hydroxy compounds
- Sulfate amount in body is limited
- Leads to water soluble and inactive metabolites
- Glucuronidation of phenols is a competing reaction and may predominate

Sulfate Conjugation



PAP: 3'-phosphoadenosine- 5'-phosphate



R = H Albuterol

R = SO_3H Metabolite

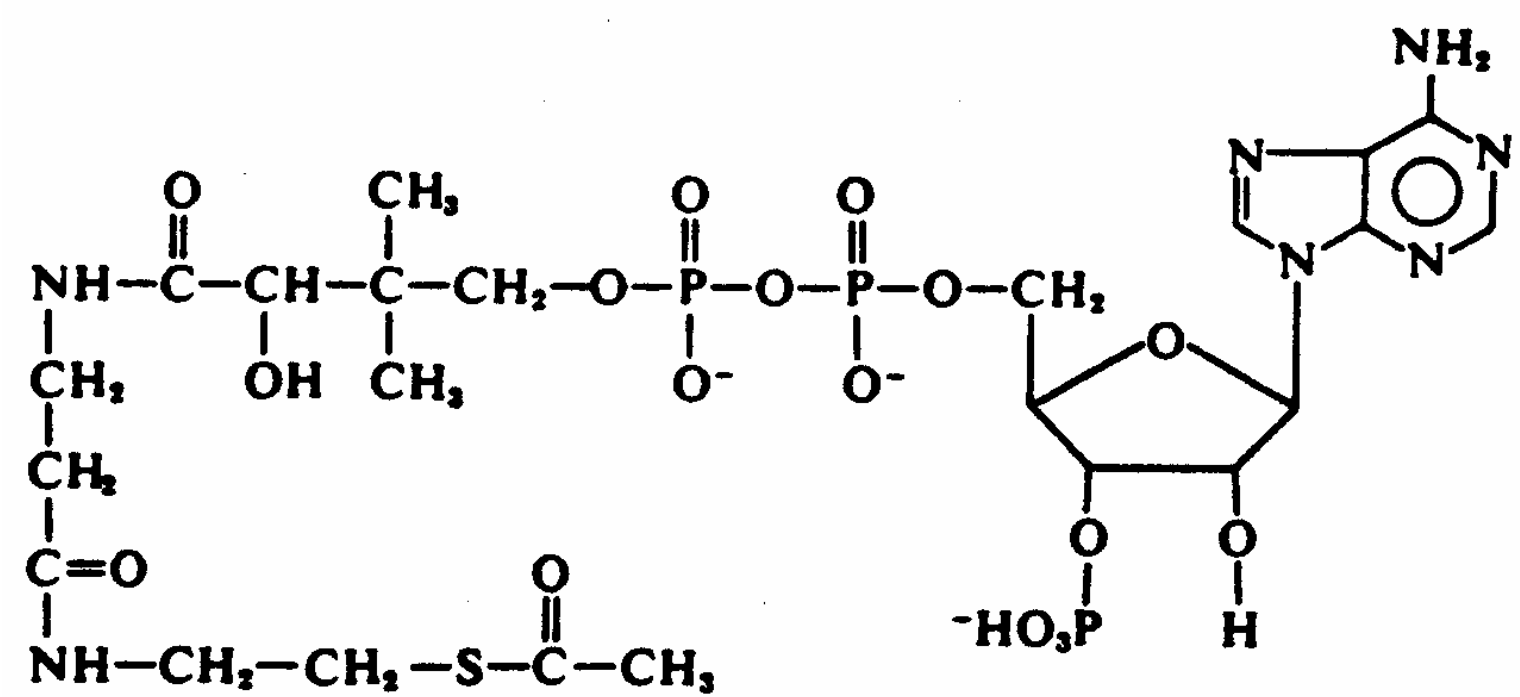
Acetylation

N-Acetyltransferase

- A soluble enzyme
- Isoniazid is a substrate
- Genetic variation occurs
  - Some individuals are fast acetylators
  - Some individuals are slow acetylators
- Acetyl coenzyme A is the endogenous donor molecule

- Important metabolic route for drugs containing primary amino groups
- Aromatic amines ($ArNH_2$), sulfonamides ($H_2NC_6H_4SO_2NHR$),
- Hydrazine ($-NHNH_2$), hydrazides ($-CONHNH_2$) and primary aliphatic amines
- Gives inactive and nontoxic metabolites but does not enhance water solubility much

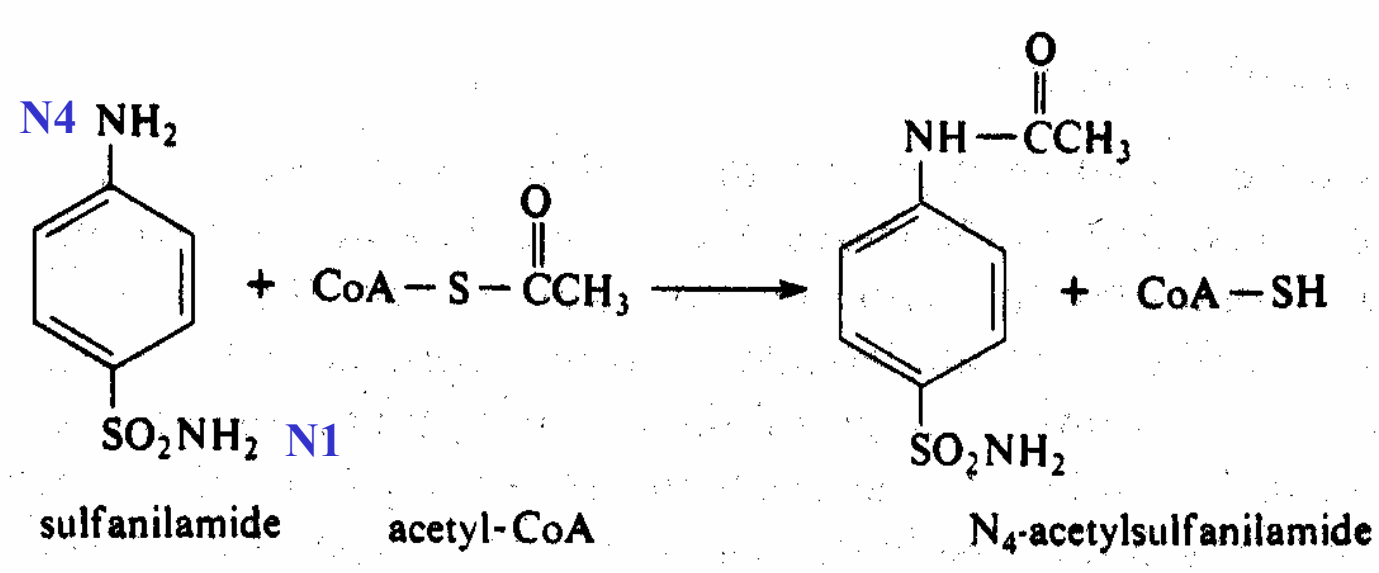
Acetyl CoA



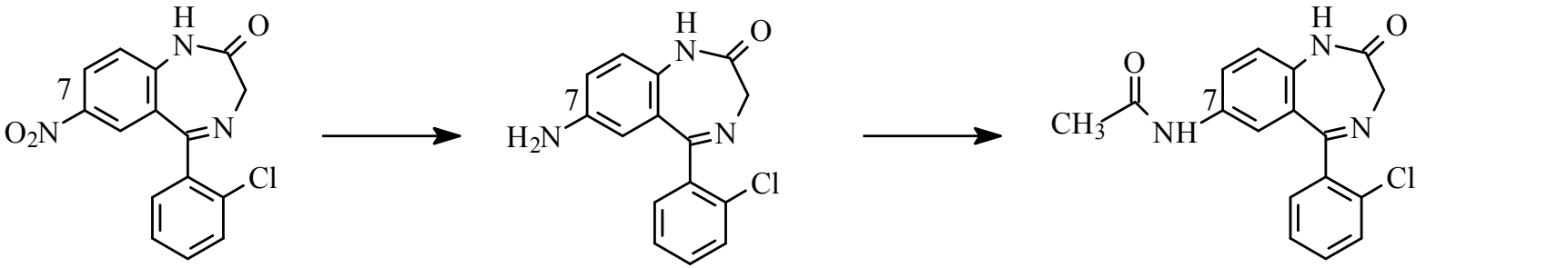
•Various acetylases, for examples, choline acetylase and N-acetyl transferase, all soluble enzymes, conduct the transfer of the acetyl group of acetyl CoA to various substrates.

•For example, N-acetylation of isoniazid. Genetic polymorphism occurs with N-acetyltransferase.

N-Acetyltransferase



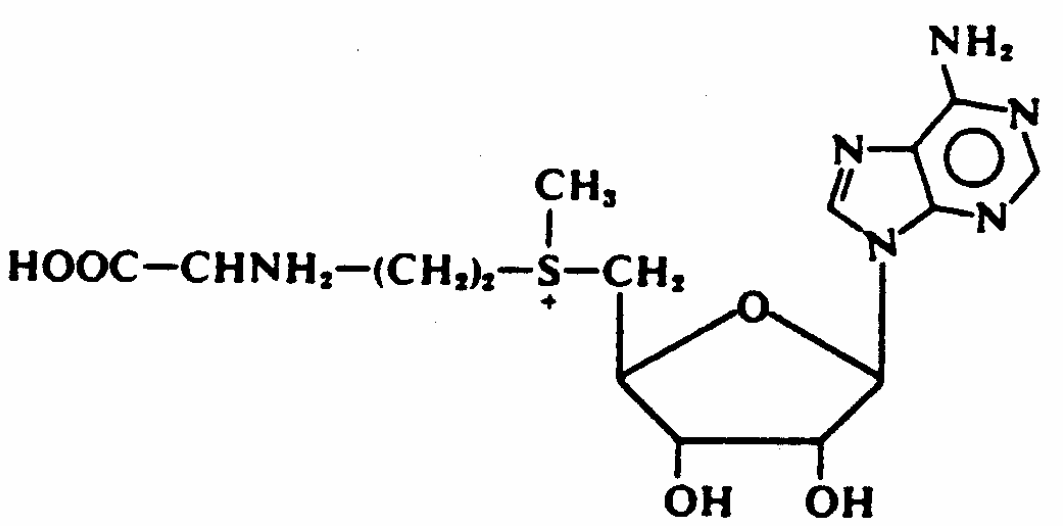
(Antibacterial)



(anticonvulsant,
muscle relaxant)

Methylation

S-Adenosylmethionine (SAM)



✓ Cytosolic enzymes such as catechol-O-methyl transferase (COMT) and phenylethanolamine-N-methyl transferase (PNMT) conducts the donation of the methyl group from the endogenously synthesized SAM to various substrates to form methylated conjugates.

✓ Norepinephrine is N-methylated by PNMT to form epinephrine. Norepinephrine, epinephrine, dopamine, and L-DOPA are O-methylated by COMT.

Methyltransferases

- A family of soluble enzymes that conducts
  - N-methylation; **N-CH_3**
  - O-methylation; **O-CH_3**
  - S-methylation; **S-CH_3**
- S-adenosylmethionine (SAM)is the endogenous donor molecule. It is demethylated to S-adenosylhomocysteine

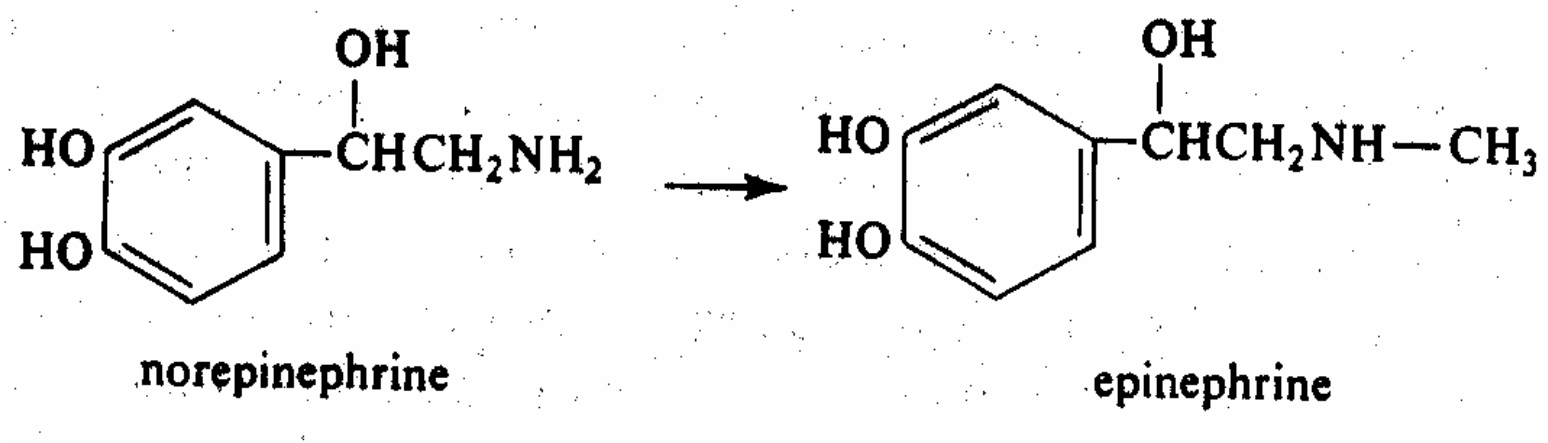
Methylation

- ✓ Methylation reactions play an important role in biosynthesis of many endogenous compounds and inactivation of numerous active biogenic amines
- ✓ Minor pathway for conjugation of drugs and xenobiotics
- ✓ Does not give polar, water soluble metabolites but pharmacologically inactive products
- ✓ Catechols, phenols, amines and N-heterocyclic and thiol compounds
- ✓ Substrates undergoing O-methylation by COMT must contain an aromatic1,2-dihydroxy group

N-Methyltransferases

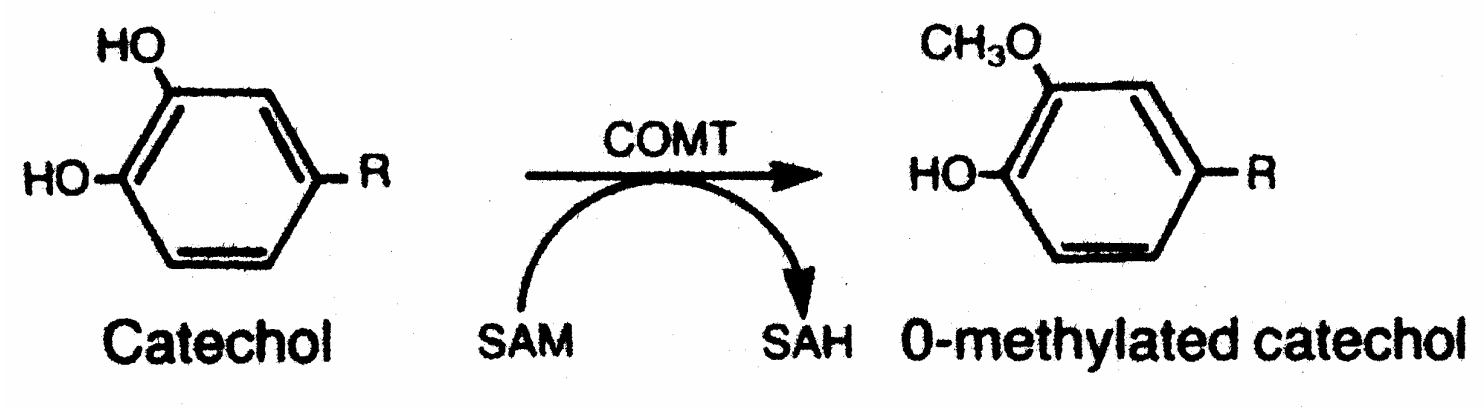
PNMT- Phenylethanolamine-N-methyltransferase

Norepinephrine $\xrightarrow[\text{SAM}]{\text{PNMT}}$ Epinephrine



(Neurotransmitters)

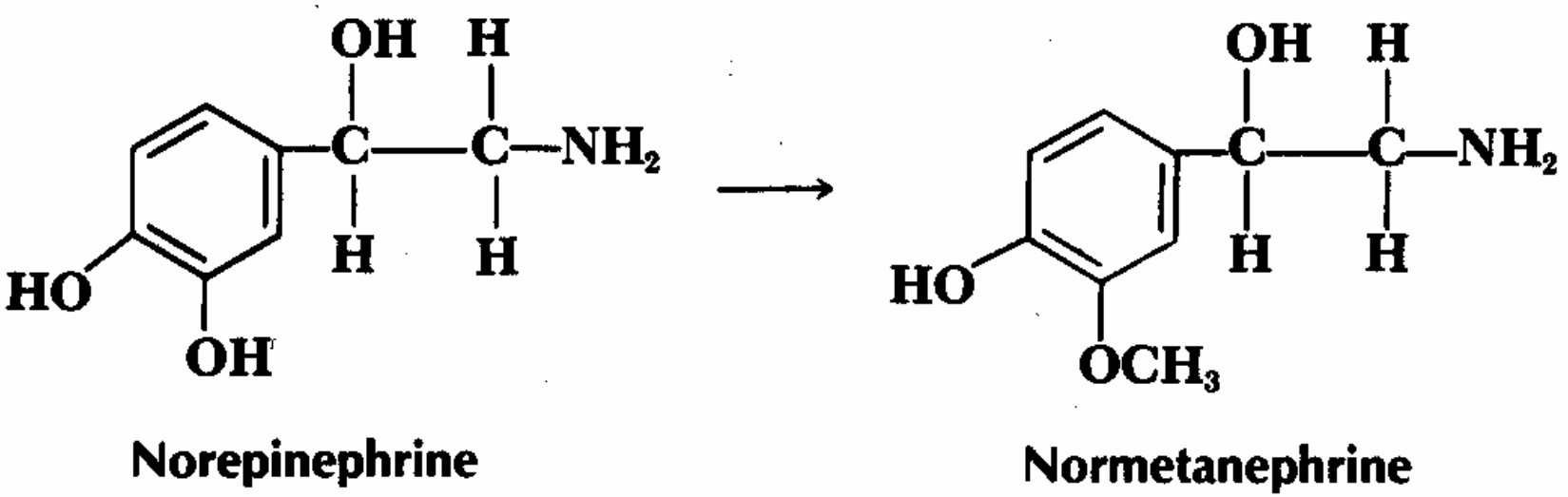
O-Methylation Of Catecholamines



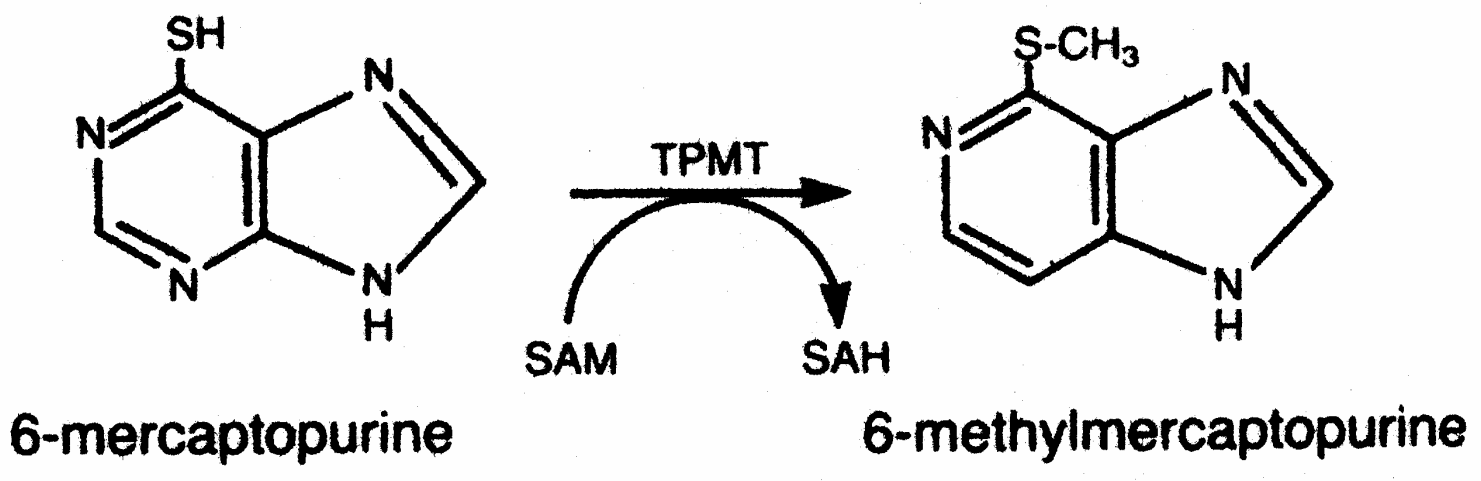
COMT- catechol-O-methyltransferase

O-Methylation of Norepinephrine

COMT- catechol-O-methyltransferase



S-Methylation of 6-Mercaptopurine

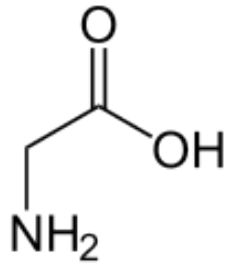


(Immunosuppressive)

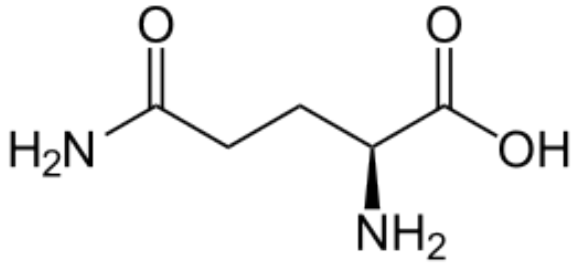
TPMT - thiopurinemethyltransferase; some individuals are deficient in this enzyme that is critically important for the metabolism of this agent

AMINO ACID CONJUGATION

- ✓ Amino acids, glycine and glutamine are used to conjugate carboxylic acids, particularly aromatic acids and arylalkyl acids
- ✓ Carboxylic acid substrate is activated with ATP and coenzyme A (CoA) to form an acyl-CoA complex
- ✓ Limited amount of amino acids in body is available so few conjugation reactions occur
- ✓ Competes with conjugation with glucuronic acid
- ✓ Polar and water soluble metabolites
- ✓ Glycine conjugation occurs with aromatic acids and arylalkyl acids
- ✓ Glutamine conjugation occurs with arylacetic acids

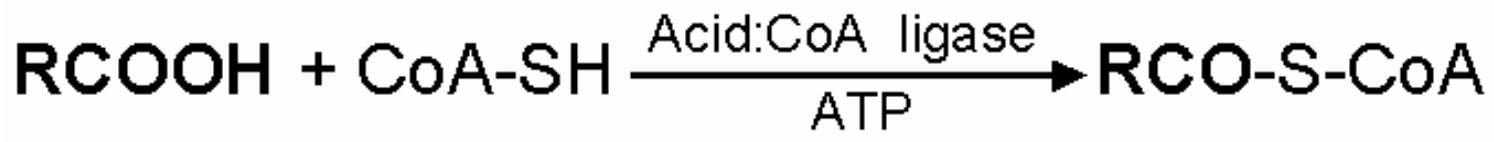


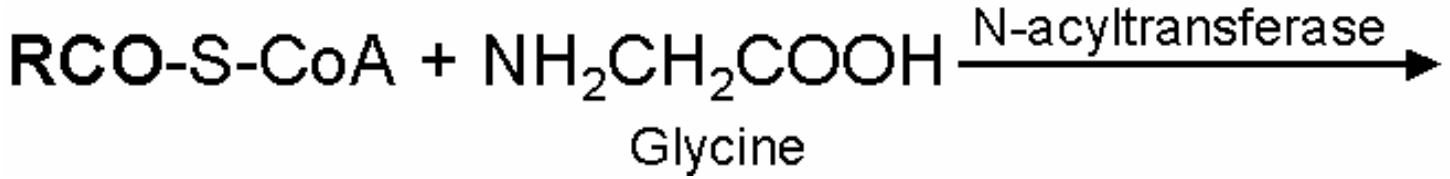
Glycine

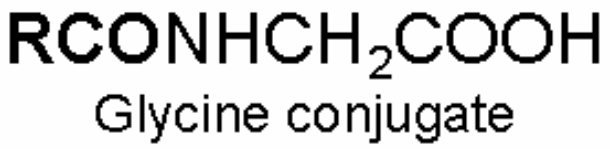


Glutamine

AMINO ACID CONJUGATION



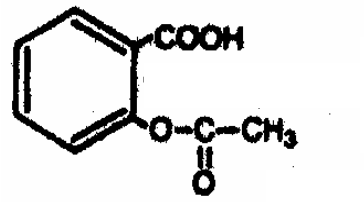




(mitochondria)

Salicyluric Acid is the Glycine Conjugate of Aspirin

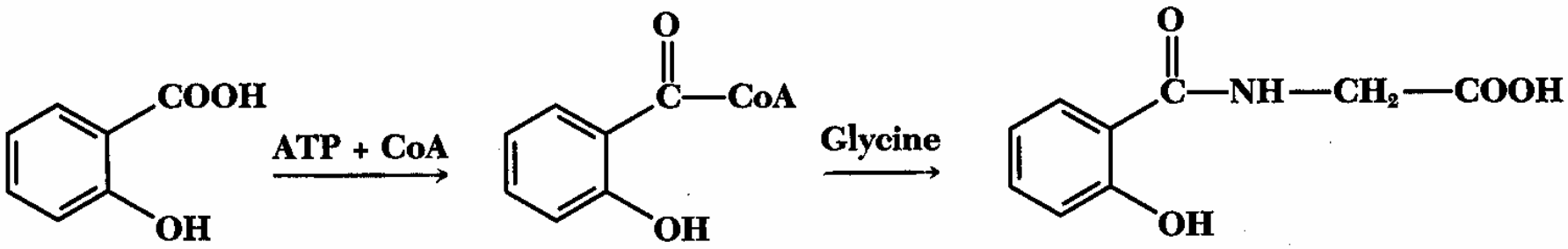
✓ Multiple Metabolic Pathways Exist for Aspirin's Metabolism



Aspirin (ASA)

• Hydrolysis produces salicylic acid metabolite

Amide linkage

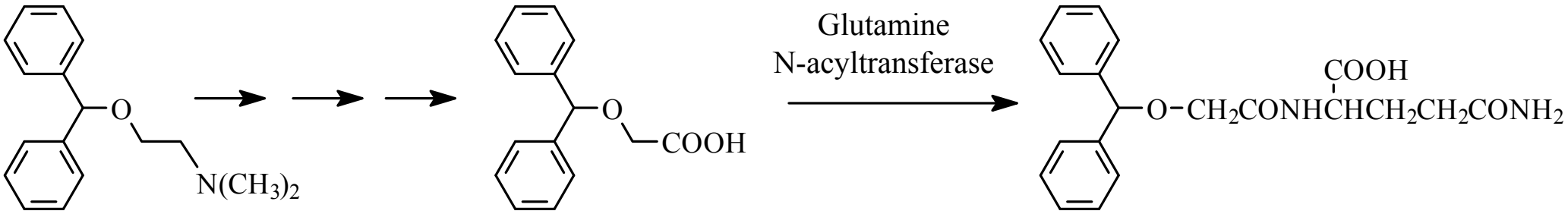


Salicylic acid **Salicyl—CoA** **Salicycluric acid**

➢ Salicyluric acid, the glycine conjugate of salicylic acid, is the main metabolite of aspirin

➢ Approximately 76% of aspirin is metabolized through amino acid conjugation.

Glutamine Conjugation

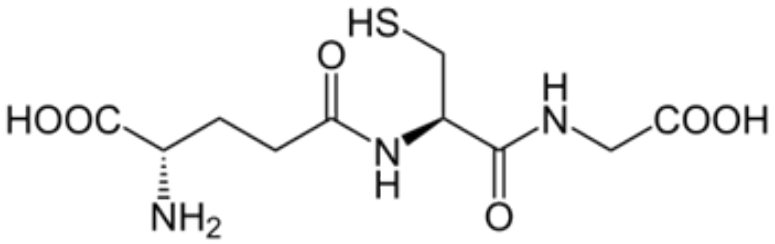


Diphenhydramine

Glutamine conjugate

Glutathione (GSH) Conjugation

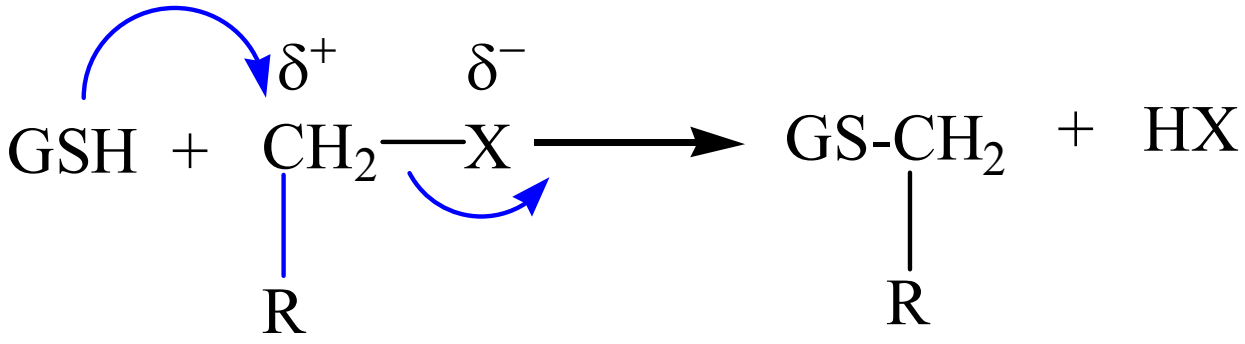
- Important pathway for detoxifying chemically reactive electrophilic compounds
- Covalent interaction of metabolically generated electrophilic intermediates with cellular nucleophiles leads to drug toxicity
- GSH protects cellular constituents by bonding to metabolites via –SH group



Glutathione

- Xenobiotics conjugated with GSH usually are not excreted as such but undergo further biotransformation to give S-substituted N-acetylcysteine products called mercapturic acid
- Nucleophilic GSH reacts with electrophilic substrates
  - * nucleophilic displacement at an electron deficient carbon or heteroatom
  - * nucleophilic addition to an electron deficient double bond

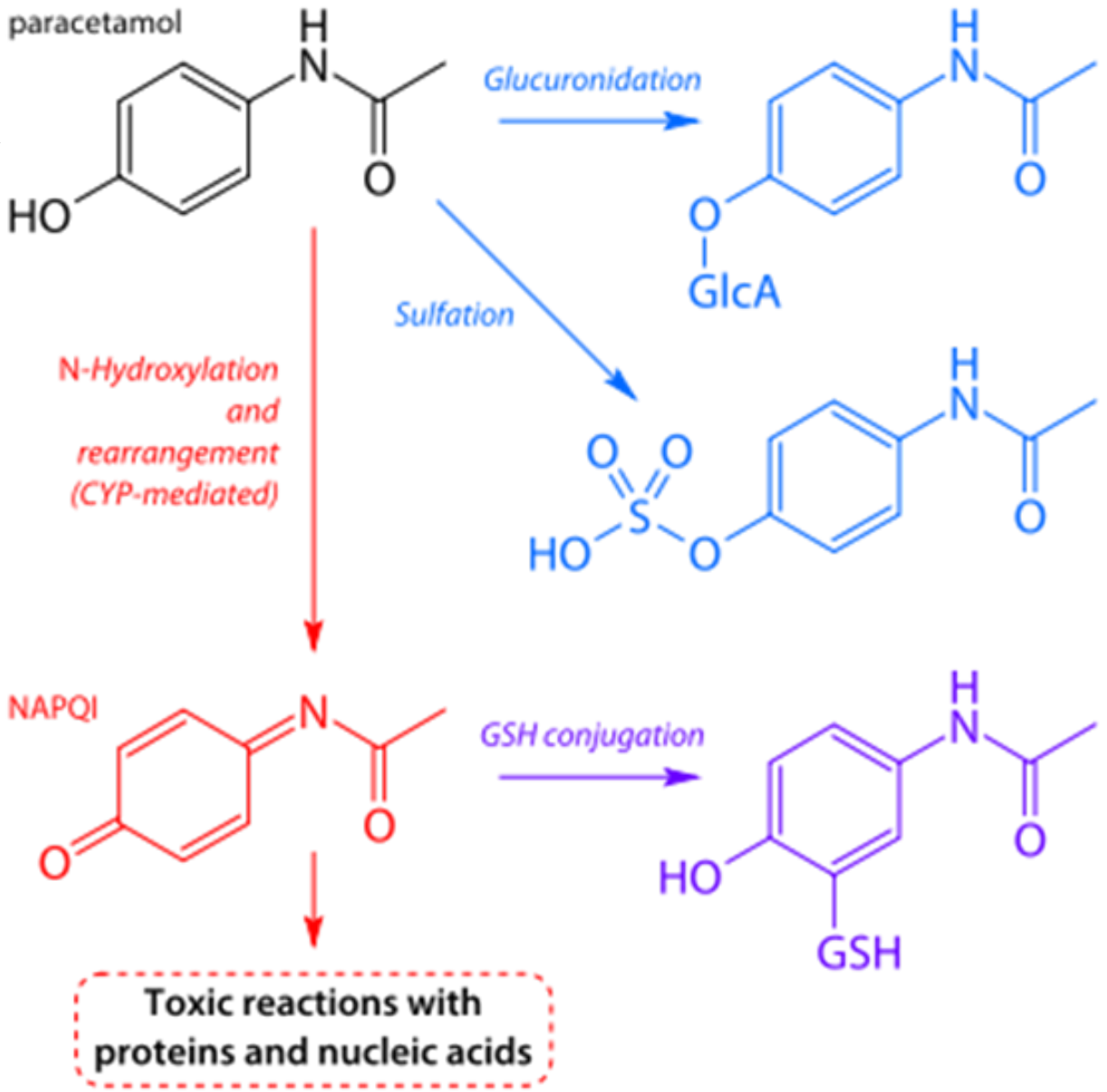
- Aliphatic and arylalkyl halides (Cl, Br, I), sulfates (OSO_3^-), sulfonates (OSO_2R),
- Nitro compounds (NO_2), and organophosphates ($OP[OR]_2$) have electron deficient carbon atoms to conjugate with GSH
- If not sufficiently electron deficient (not enough electron withdrawing groups) GSH conjugation does not take place



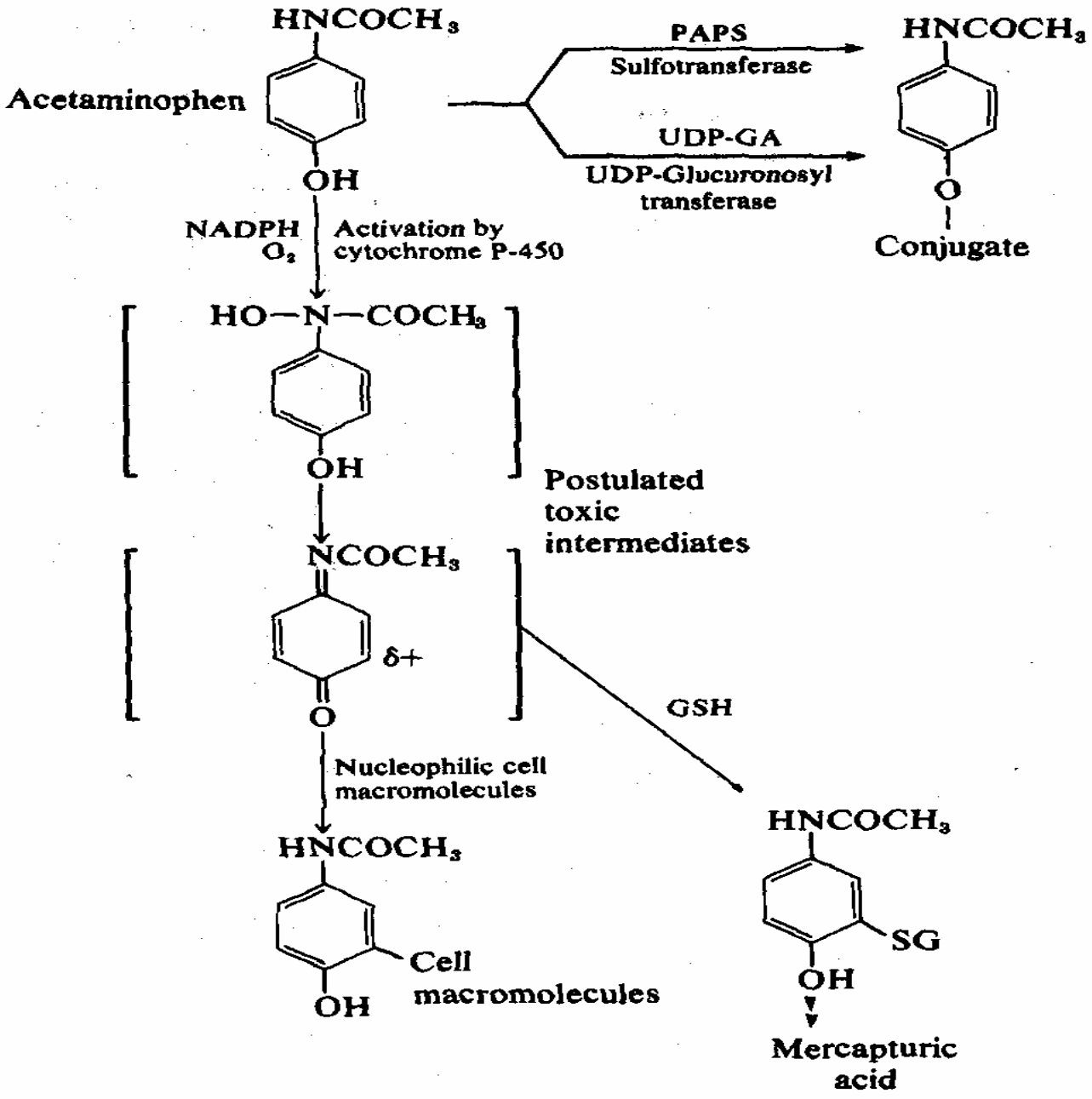
R= Alkyl, aryl, benzyl, allylic
X = Br, Cl, I, OSO_3^-, OSO_2R, $OPO(OR)_2$

➢ Paracetamol is metabolised primarily in the liver, into non-toxic products

acetaminophen



Bioactivation of Acetaminophen

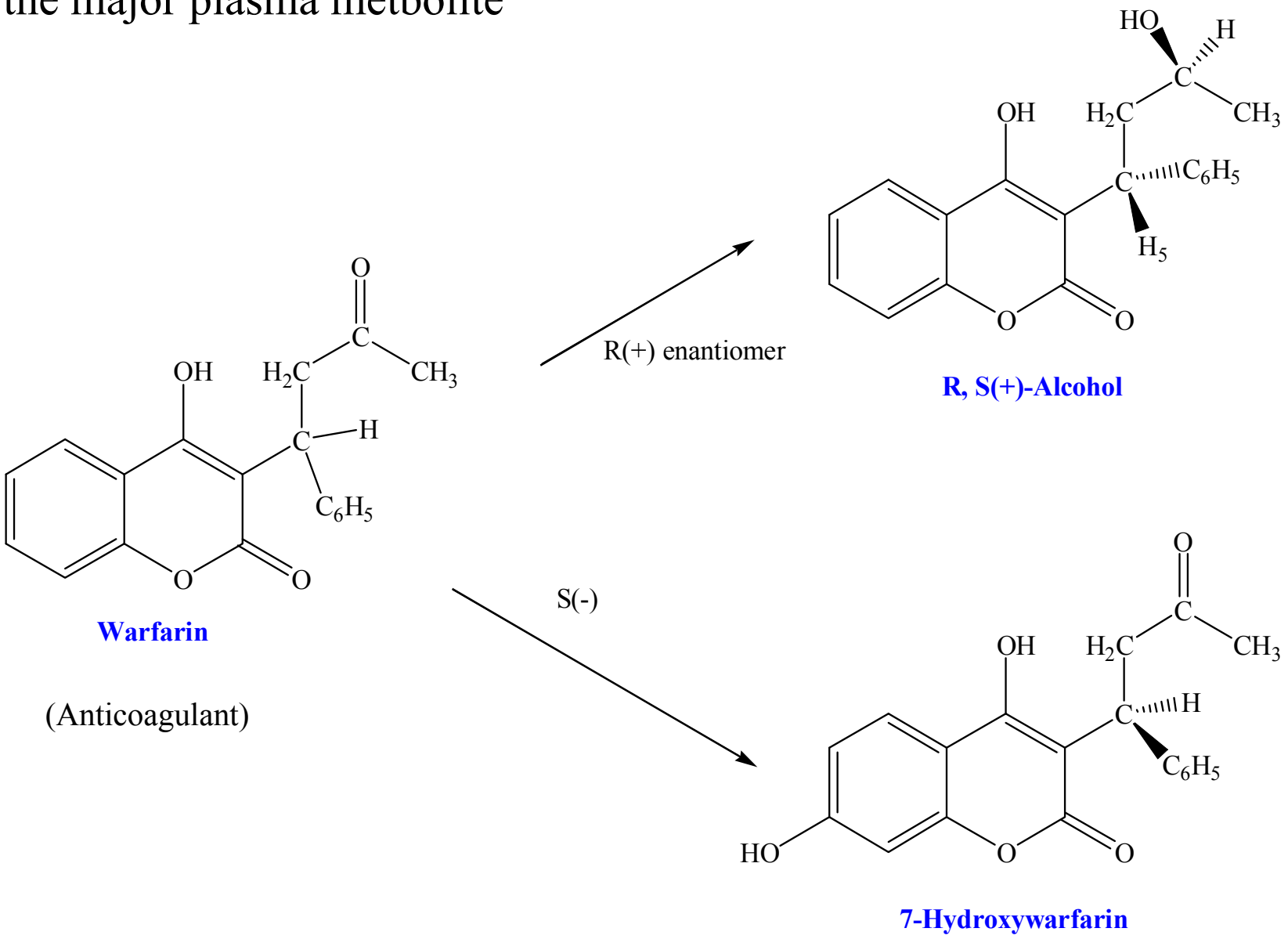


Stereochemical Aspects of Drug Metabolism

- Many drugs often are administered as racemic mixtures in humans
  e.g. warfarin, propranolol, hexobarbital, ibuprofen, glutethimide
- May differ in pharmacological activity
- Individual enantiomers of a racemic drug often are metabolized at different rates and could be metabolized by different pathways
- Substrate stereoselectivity: a preference for one stereoisomer as a substrate for a
- Metabolizing enzyme or metabolic process
- Biotransformations could lead to new asymmetric center e.g. bioreduction of ketone xenobiotics

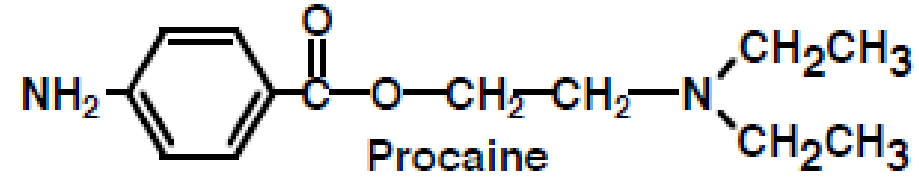
Metabolism of Warfarin Enantiomers:

- ✓ More active (S)(-) isomer is 7-hydroxylated (aromatic hydroxylation)
- ✓ (R)(+) isomer undergoes keto reduction to yield (R,S) warfarin alcohol as the major plasma metbolite



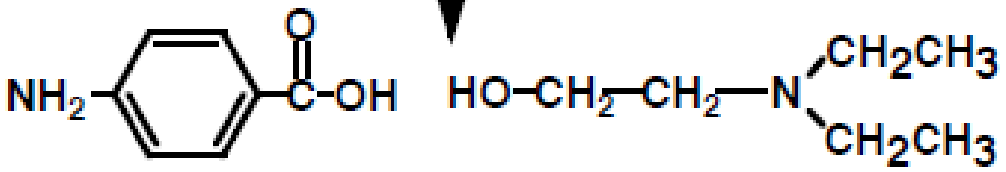
Importance of Drug Metabolism

- Metabolism => Termination of Drug Action
  - Bioinactivation



Procaine

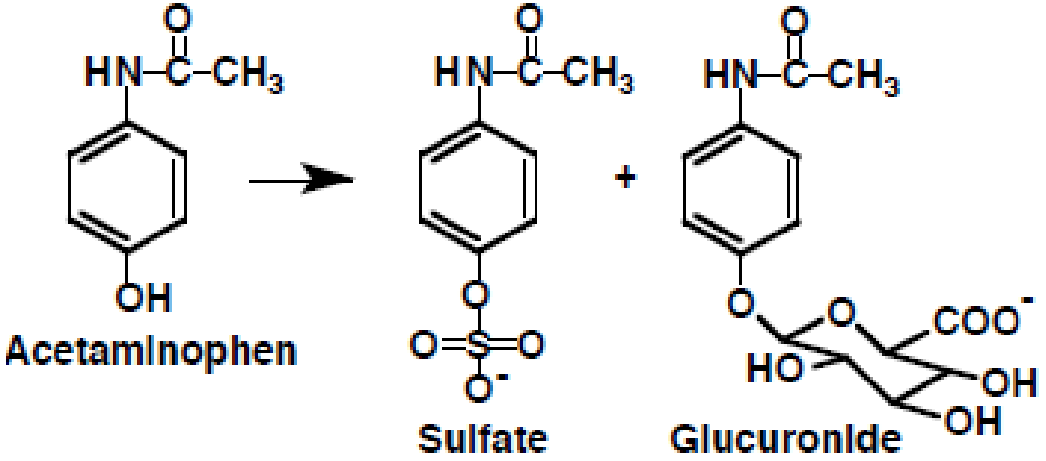
*local anesthetic



- hydrolysis

Importance of Drug Metabolism

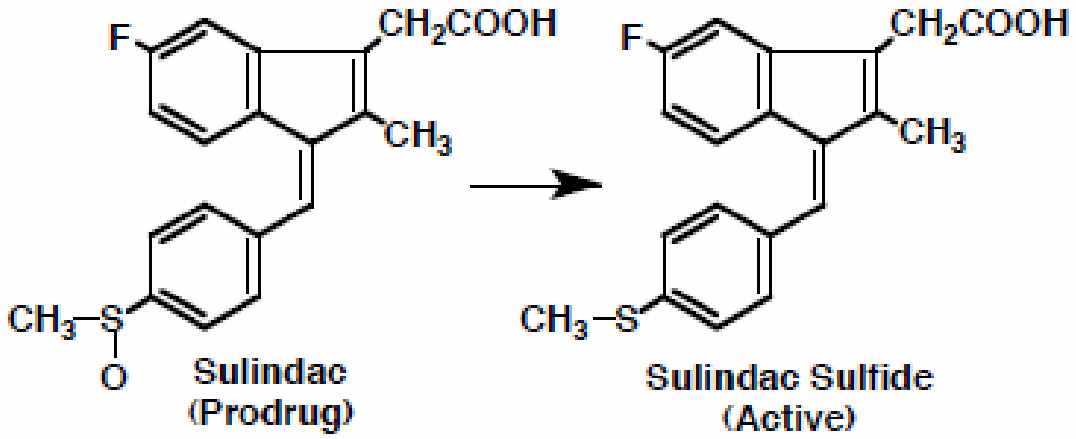
- Metabolism => Termination of Drug Action
  - Elimination (water soluble).



- Conjugation

Importance of Drug Metabolism

- Metabolism => Bioactivation
  - Prodrugs (by Design!)

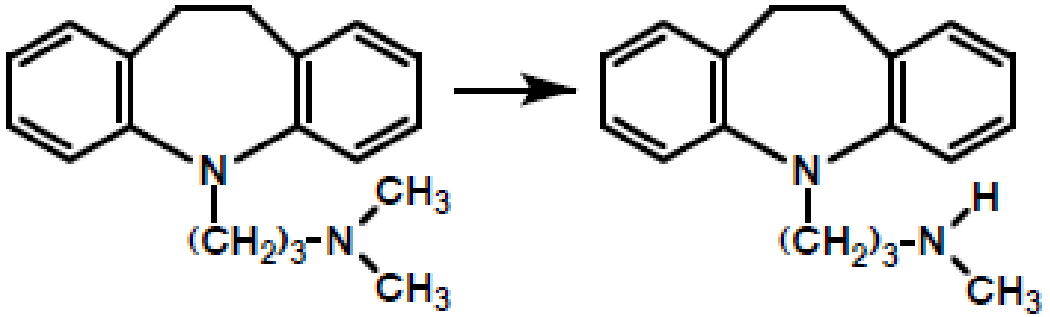


*non-steroidal anti-inflammatory drug

- Sulfoxide to sulfide reduction

Importance of Drug Metabolism

- Metabolism => Bioactivation
  - Active Metabolites (Surprise! :)



Imipramine **Desipramine**

*antidepressant

- N-demethylation

Meperidine (ethyl 1-methyl-4-phenylpiperidine-4-carboxylate) is a narcotic analgesic drug which undergoes phase 1 and phase 2 reactions. Show its metabolites forming from possible metbolism pathways.