Drug metabolism

Metabolism:-

Metabolism is defined as the sum total of all chemical reactions that occur in the body. (or) conversion of from one chemical form to another.

Drug metabolism:-

Enzymatic chemical reactions that are responsible for the conversion of drugs into metabolites with in the body.

→ Drug metabolism / Biotransformation refers to modification of xenobiotic in the biological system.

Xenobiotics:- All the chemical substances that are not nutrients which enter into the body through ingestion, inhalation/absorption is called "Xenobiotics / Exgeneous compounds"

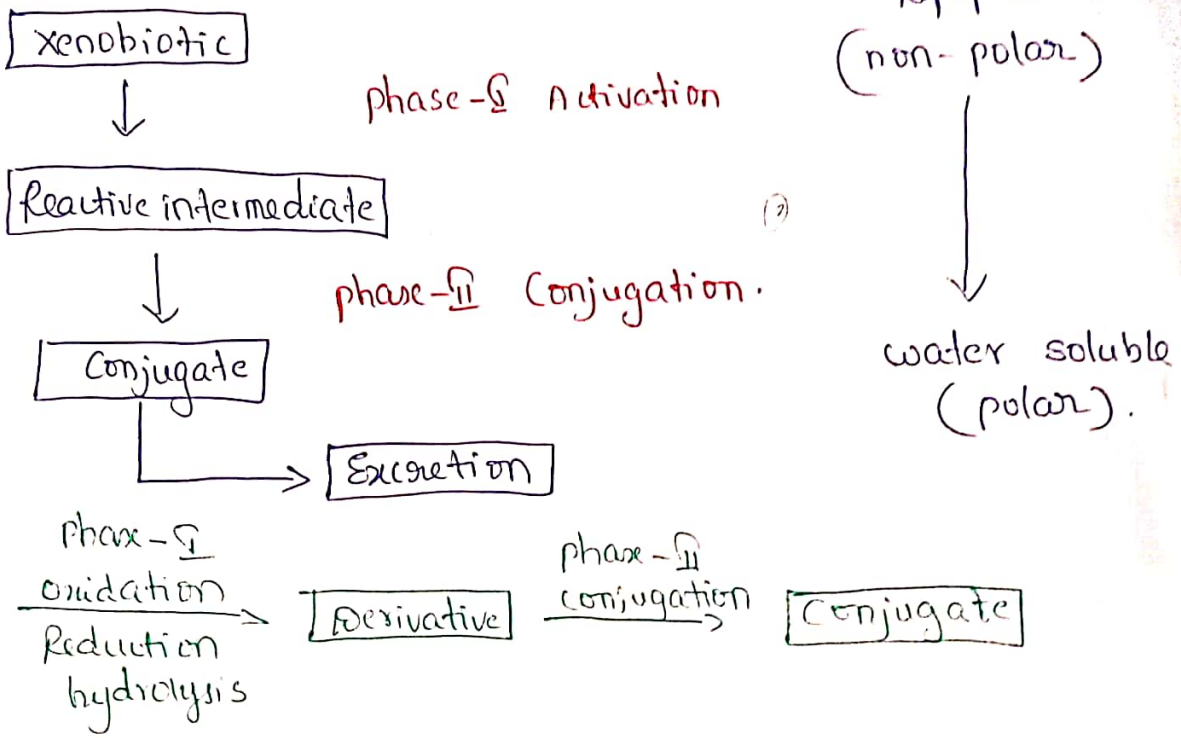
* Drug biotransformation is a detoxification process.

→ Water soluble agents undergo renal excretion whereas lipid soluble substances are passively reabsorbed from the renal tubule into the blood after glomerular filtration.

→ If this phenomenon continues, the drug would accumulate in the body and precipitate toxic reaction.

→ To prevent this, the body is armed with the metabolic system which transforms the water insoluble lipophillic non-polar drug into polar & water soluble product that can easily excreted by kidney.

Converting lipophilic to water soluble compounds.



Aims of drug metabolism:—

* To convert active drug into inactive substance.
* To convert active drug into active metabolite. Eg: codeine – morphine
  diazepam – oxazipam
* To convert inactive drug into active drug. Eg: L-Dopa – Dopamine.
* To convert active drug to toxic substances. Eg: paracetmol – N-acetyl
  benzquin

Sites of Bio-transformation

Organ sites of drug metabolism :-

liver
small intestine
kidney (proximal tubule)
skin (epithelial cells).
lungs (type II cells)
plasma
All organs of the body.

* Drug metabolism occurs in all the tissues, but the principle site is the *Liver* (microsomal enzyme system of hepatocytes).

Cellular sites of drug metabolism.

cytosol
Mitochondria
Lysosomes
Smooth endoplasmic reticulum (microsomes).

- orally administered drugs are absorbed through mucous membran of the small intestine or from the stomach.
- Once out of GI tract it is carried by the blood stream to the liver, where it is usually first metabolised.

* Metabolism by liver enzyme prior to the drug reaching the Systemic circulation is called *first-pass metabolism / pre-Systemic effect / first-pass Effect*; which may result in the complete deactivation of drug.

(4) Drug metabolising Enzymes :-

-> A number of enzymes in animals are capable of metabolising drugs. These enzymes are located mainly in the liver, but may also be present in other organs like lungs, kidney intestine, brain plasma etc.

-> Majority of drugs are acted upon by relatively non-specif enzymes, which are directed to types of molecules rather than to specific drugs.

-> The drug metabolising enzymes can be broadly divided into two groups

i) Microsomal

ii) non-microsomal enzymes.

Microsomal enzymes :- The endoplasmic reticulum of liver and other tissues contain a large variety of enzymes, together called microsomal enzymes.

-> They catalyse glucoronide conjugation, most oxidative reactions and some reductive & hydrolytic reactions.

Eg :- Monooxygenases, glucoronyl transferase etc.
alcohol dehydrogenases, aldehyde dehydrogenases.

Non-microsomal enzymes :— Enzymes occurring in organells/ sites other than endoplasmic reticulum (microsomes) are called non microsomal enzymes.

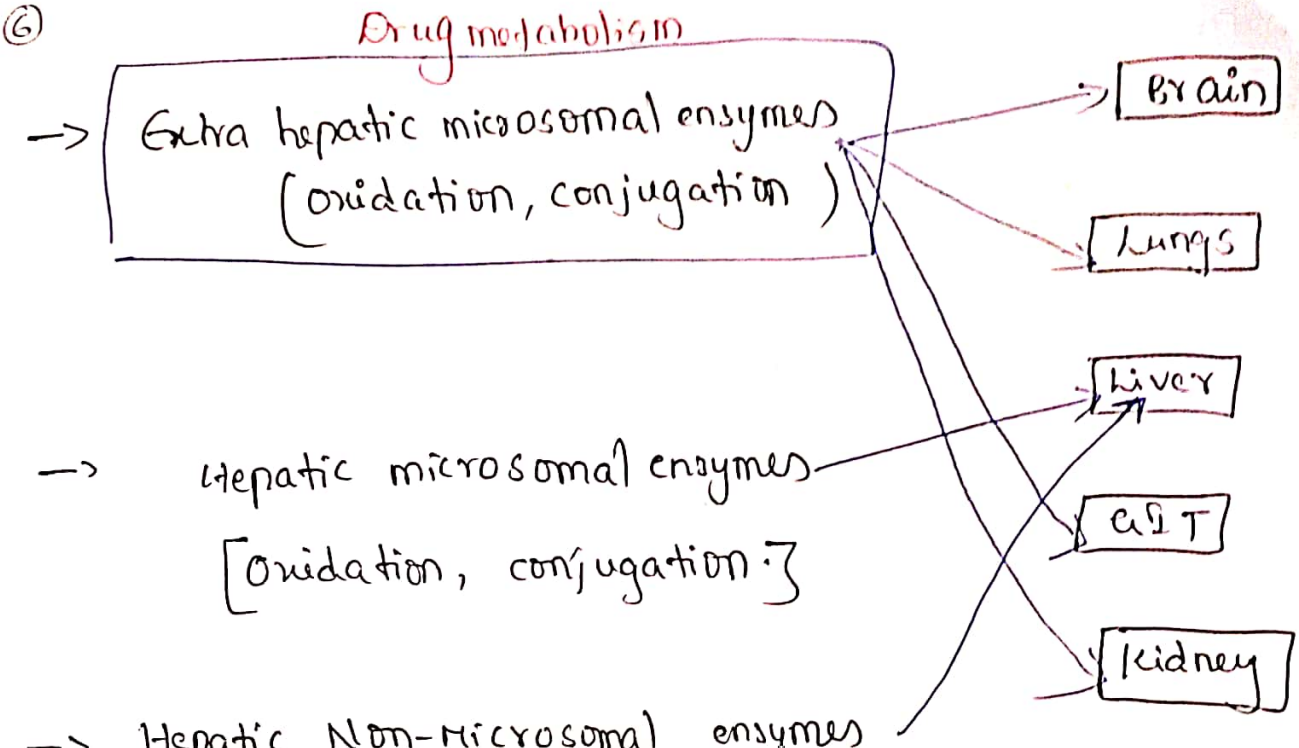
- These are usually present in the cytoplasm, mitochondria etc.
- They are usually non-specific enzymes that catalyse few oxidative reactions, a no. of reductive and hydrolytic reactions, and all conjugate reactions other than glucuronidation.

Xenobiotics Metabolising enzymes.

Enzymes	Reactions.
phase I "Oxygenases"	
cytochrome P450s (P450 or CYP)	C & O oxidn, dealkyln, others
Flavin-containing monooxygenases (FMO)	N, S & P oxidation
Epoxide hydrolases (mEH, sEH)	Hydrolysis of epoxides.
Phase-II "Transferases"	
Sulfotransferases (SULT)	Addition of sulfate
UDP-Glucuronosyl transferases (UGT)	Addn of glucuronic acid
Glutathione-S-transferases (GST)	" " Glutathione
N-Acetyl transferases (NAT)	Addn of acetyl group.
Methyl transferases (MT)	" " methyl group.
Other enzymes :—	
Alcohol dehydrogenases	Reduction of Alcohols.
Aldehyde dehydrogenases	" " aldehydes.
NADPH-quinone oxidoreductase (NQO)	" " Quinones.

⑥

Drug metabolism



→ Hepatic microsomal enzymes
[Oxidation, conjugation]

→ Hepatic Non-Microsomal enzymes
[acetylation, sulfation, GSH, alcohol/aldehyde dehydrogenase, hydrolysis, ox/red n]

Types of Drug metabolism/Biotransformation.

phase-I reactions

- non synthetic phase
- A change in drug molecule generally results in the intdn of a functional grp into molecules or exposure of new functional grp's of molecules.

Reactions:- oxidation
reduction
hydrolysis.

- In phase-I reactions, small polar functional groups like $-OH$, $-NH_2$, $-SH$, $COOH$ are either added or unmasked, on the lipid soluble drugs, so that the resulting products may undergo phase II reactions.

* result in activation, change (or) inactivation of drug.

phase-II reactions.

* synthetic phase.
* last step in detoxification reactions and almost always result in lots of biological activity of a compound.
* involves conjugation of functional groups of molecules with hydrophilic endogeneous substrates - formation of conjugates
* involve attachement of small polar endogeneous molecules like glucuronic acid, sulphate, methyl, amino acids et to their uncharged drugs/ phase I products.
* products called as conjugates are coater-soluble metabolites, which are readily excreted from the body.

Phase I Reactions :—

I. Oxidative Reactions

i) Oxidation of aromatic moieties

ii) Oxidation of olefins

iii) Oxidation of benzylic, allylic 'c' atoms & carbon atoms α to carbonyl and imines

iv) Oxidation of aliphatic & alicyclic carbon atoms.

v) Oxidation of involving carbon-heteroatom system.

vi) Carbon-Nitrogen systems (oxidative deamination, N-oxide form^n, N-Hydroxylation)

vii) Carbon-oxygen system (O-dealkylation)

viii) Carbon-sulfur system (S-dealkylation, S-oxidation, and Desulfuration)

ix) Oxidation of alcohols & aldehydes.

x) Other miscellaneous oxidative reactions.

II Reductive reactions.

i) Reduction of aldehydes & ketones

ii) Reduction of NitRO & azo compounds.

III. Hydrolytic Reactions :—

i) Hydrolysis of esters & amides

ii) Hydration of epoxides & arene oxides

phase II reactions :-

i) Glucuronic acid conjugation
ii) Sulfate conjugation
iii) conjugation with glycine, glutamine & otheramino acids.
iv) glutathione / mercapturic acid conjugation.
v) Acetylation
vi) Methylation.

phase I Reactions.

Oxidative reactions :-

-> Oxidative reactions are most important metabolic reactions.
-> There are imp. for drugs because they increase hydrophilicity of drugs by introducing polar functional groups such as -OH
-> Oxidation of drugs is non-specifically catalysed by a number of enzymes located primarily in the microsomes.

* Oxidation by cytochrome P450 isoenzymes
* Oxidation by enzymes other than cytochrome P450 (non-microsomal)
    a) oxidn of alcohol by dehydrogenase
    b) " " aldehyde by aldehyde dehydrogenase
    c) N-dealkylation by monoamine oxidase.

* The most imp. group of oxidative enzymes are microsomal mono oxygenases or mixed function oxidases (MFO).

(10)

-> These enzymes are located mainly in the hepatic endoplasmic reticulum and require both molecular oxygen (O_2) and reducing NADPH to effect the chemical reaction.

$$RH + O_2 + NADPH + H^+ \longrightarrow ROH + H_2O + NADP^+$$

product.

* NADPH = reduced nicotinamide adenine dinucleotide phosphate.
* The most important component of mixed function oxidases is the Cytochrome P-450 because it binds to the substrate and activates oxygen.

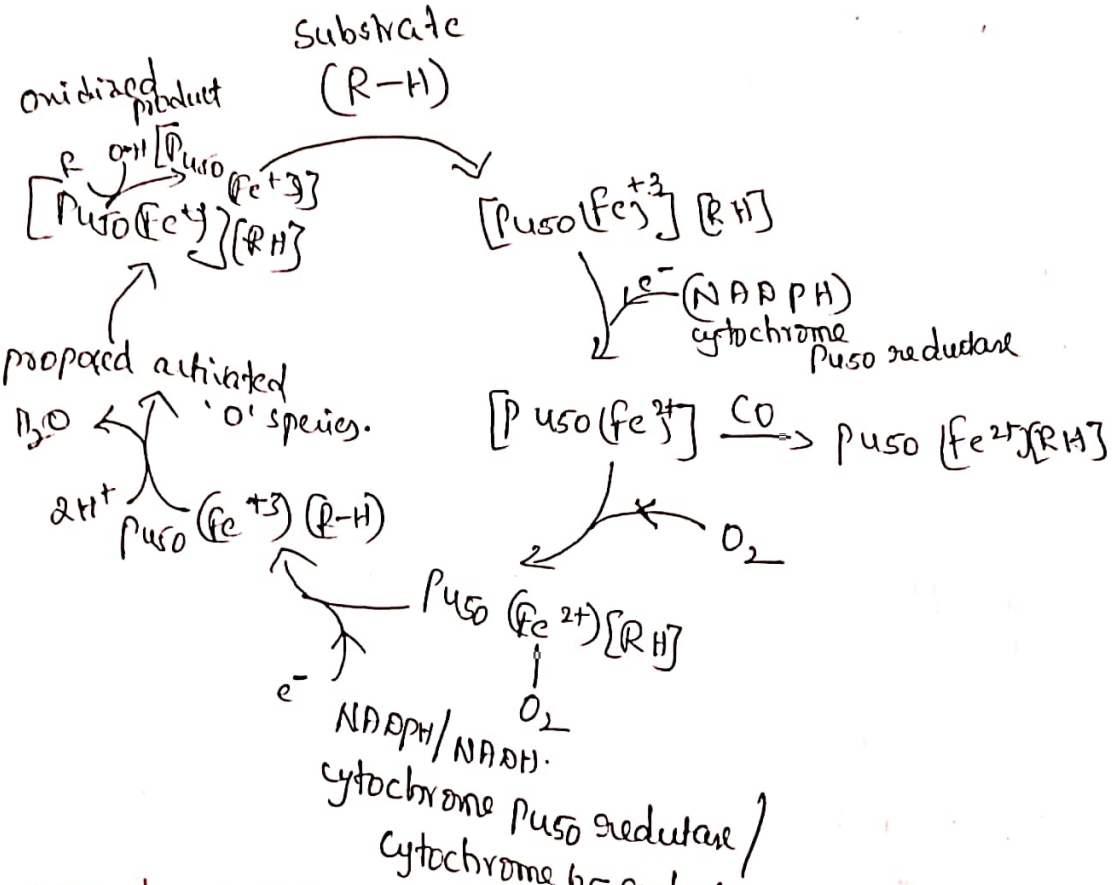
The cytochrome P450 oxidation - reduction cycle.

-> The various steps involved in the mechanism of cytochrome P450 catalysed metabolism of xenobiotics are :-

* Binding of the substrate (RH) to the oxidised form of the cytochrome P450 (Fe^{+++}) to form a complex.
* A one electron transfer from NADPH to the complex by cytochrome P450 reductase, to form reduced (Fe^{++}) P450 substrate complex this step is considered as the rate limiting step in the overall oxidation xenobiotics.
* The reduced form (Fe^{2+}) of this enzyme binds with CO to form a complex that shows maximum absorption at 450 nm hence the name cytochrome P450.
* The reduced enzyme substrate complex combines with a molecule of oxygen to form a ternary complex.
* The ternary complex combines with a 2nd electron supplied by NADH in presence of enzyme cytochrome b5 reductase to form

ternary activated oxygen P450 substrate complex.

* One atom of oxygen from the activated oxygen complex is transferred to the substrate to yield the oxidized product and the other atom forms water.
* The free oxidized form of cytochrome P450 is now ready to attach another molecule of substrate.

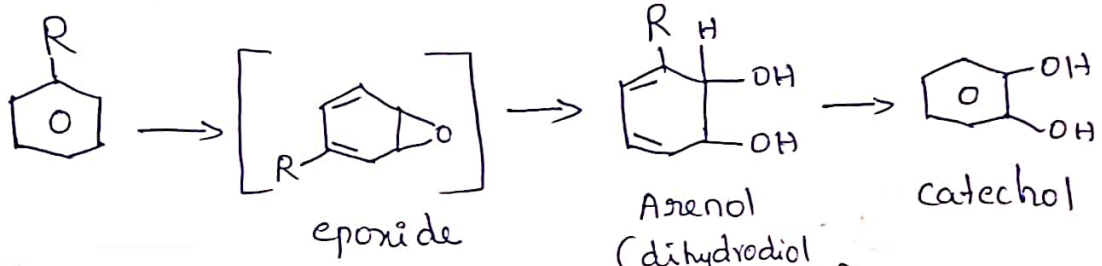


proposed catalytic reaction cycle involving cytochrome P450 in the oxidation of xenobiotics.

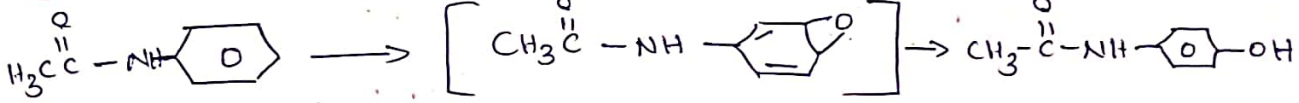
(12)

① Oxidation of Aromatic Carbon atom :-

→ The aromatic rings occurs through a reactive epoxide (Arene oxide), which can be either hydrolysed by epoxide hydrolase to a dihydrodiol or rearranged under proton catalysis to the phenol



Eg :-



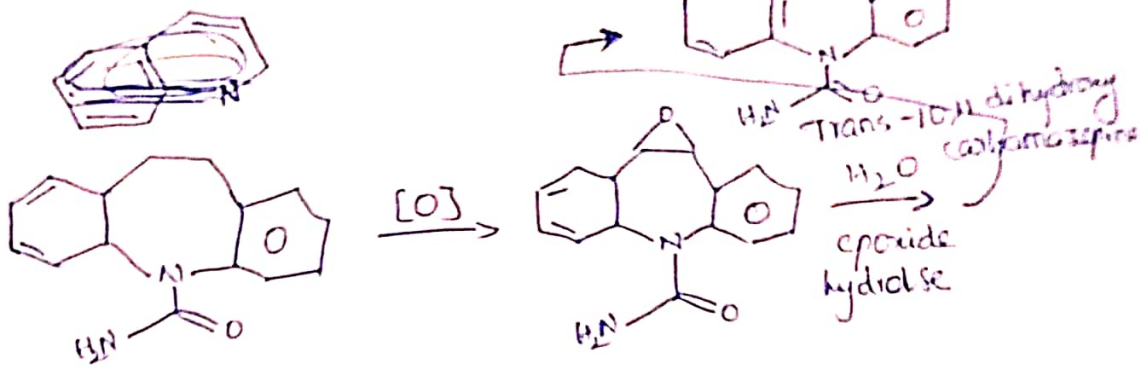
Acetanilide Arene oxide. p-hydroxy acetanilide

* Arene oxide rearranges rapidly to arenol by intermolecular hydride migration.
* Nucleophilic attack of water on the epoxide by microsomal enzyme called epoxide hydrase.

② Oxidation of olefins (C=C bond) :-

Oxidation of non-aromatic C=C ~~bond~~ double bond is analogue to aromatic hydroxylation it proceeds via epoxide to yield 1, 2 dihydrodiol. The epoxide is not very reactive like aromatic epoxide.

Eg:-

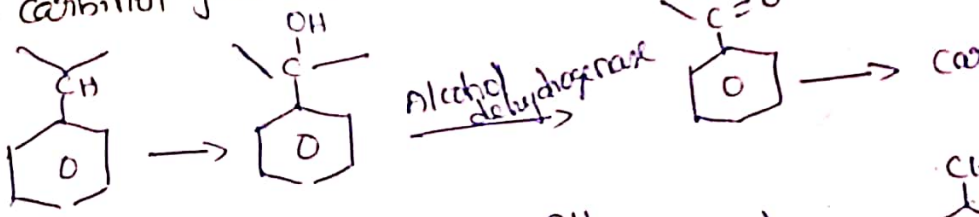


Carbamazepine

(3) **Oxidation of Benzylic carbon Atom:-**

→ carbon atom attached directly to the aromatic ring (benzylic 'c') is hydroxylated to corresponding carbinol / alcoholic metabolite.

→ alcohol metabolite further oxidized to aldehyde & further COOH & carbinol gets converted to ketone by alcohol/aldehyde dehydrogenase.



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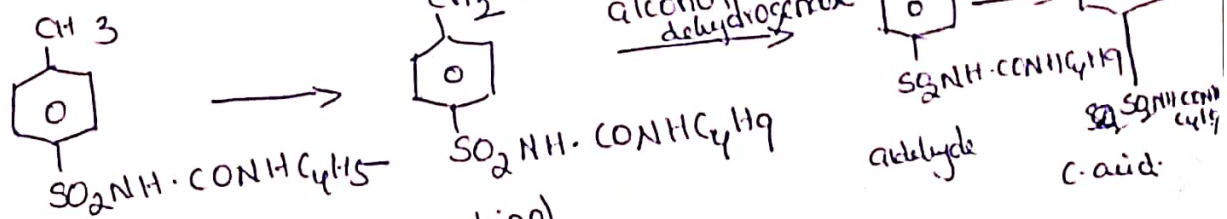


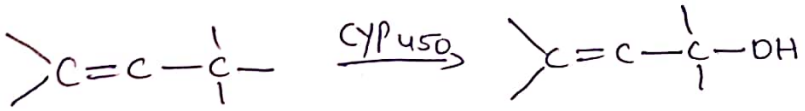
Tolbutamide.

(carbinol (alcohol).

(14)

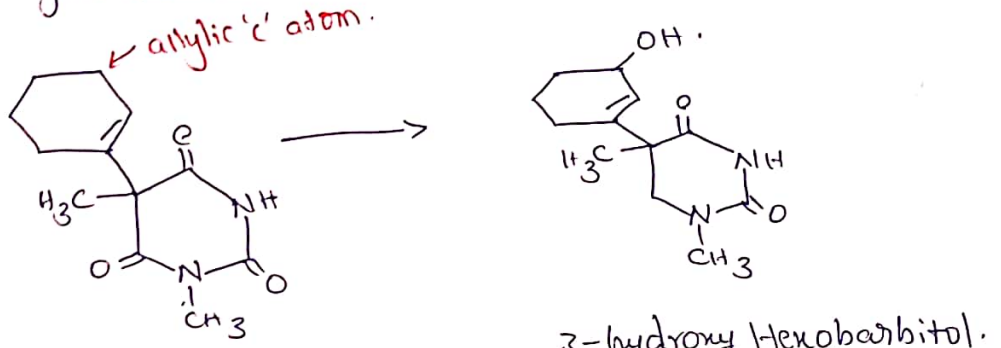
④ Oxidation of Allylic carbon atom :—

→ 'C' atom adjacent to olefinic double bond & undergoes hydroxylation similar to benzylic carbon.



allylic carbon.

Eg:



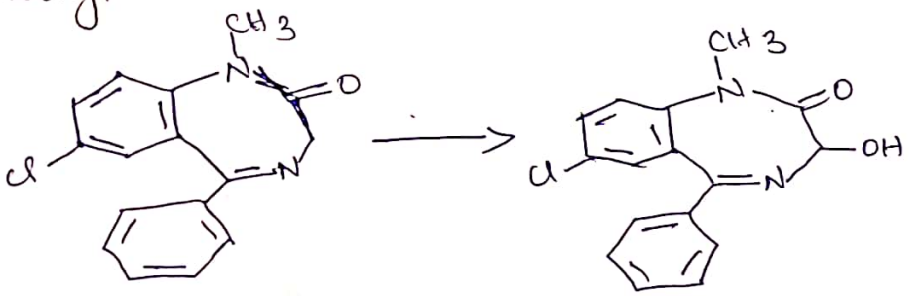
Hexobarbitol

3-hydroxy Hexobarbitol.

⑤ Oxidation of carbon atom α to Carbonyl & Imines.

The mixed function oxidase also oxidizes carbon atom adjacent to carbonyl and imino functionalities.

Eg:



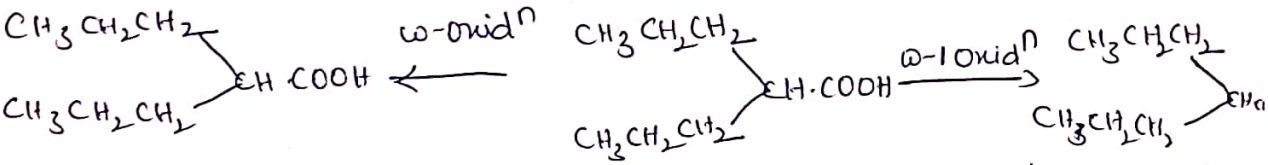
Diazepam.

3-hydroxy Diazepam.

⑥ Oxidation of Aliphatic Carbon atoms :—

Alkyl / aliphatic carbon atom can be hydroxylated at two positions, terminal methyl group is often referred as ω-oxidation & oxidation at penultimate carbon atom is called ω-1 oxidation.

$R-CH_3 \xrightarrow{[P450\ O]} R-CH_2-OH$

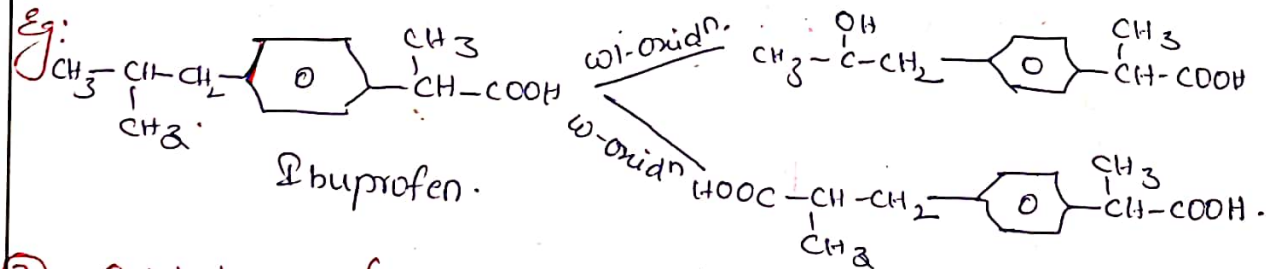


5-Hydroxy valproic acid (minor).

Valproic acid

4-hydroxy valproic acid. (major)

Eg:

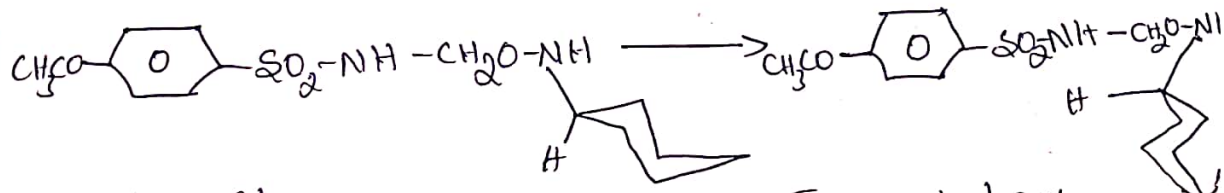


Ibuprofen.

⑦ Oxidation of Alicyclic carbon atoms :—

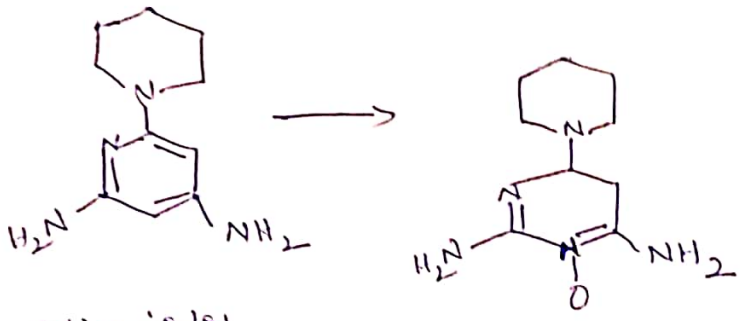
→ The cyclohexyl group is also susceptible to MFO. The enzymatic hydroxylation occurs in C-3 / C-4 leads to Cis & trans conformational stereoisomer.

Eg:



Acetohexamide.

Trans-4-hydroxy acetohexamide.



Minoxidil.

4-hydroxy minoxidil.

(5) Oxidation of Carbon-Hetero atoms.

Biotransformation of C-N, C-O, & C-S system in two ways.

i) Hydroxylation of α carbon atom attached directly to the hetero atom.

ii) Oxidation / Hydroxylation of hetero atom itself N-, S only.

A. A carbon-Nitrogen system.

1) N-Dealkylation
2) Oxidative Deamination
3) N-Oxide formation.
4) N-Hydroxylation.

B. Carbon-sulfur system.

1. S-Dealkylation
2. Desulfuration
3. S-Oxidation.

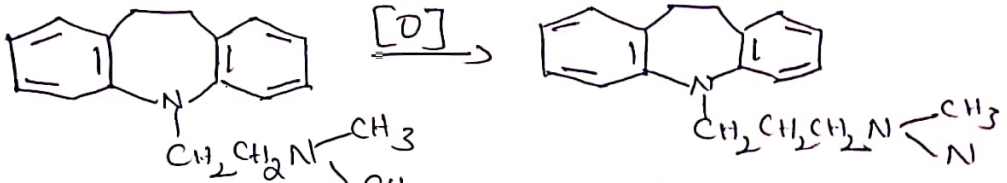
C. Carbon-Oxygen system

1. O-Dealkylation.

N- Dealkylation :-

$$R-NH-CH_3 \longrightarrow [R-NH-CH_2OH] \longrightarrow RNH_2 + HCHO.$$

Eg:



Imipramine.

Desipramine

Oxidative Deamination :-

$$R-\underset{\underset{NH_2}{|}}{CH}-CH_3 \longrightarrow \left[R-\overset{\overset{OH}{|}}{\underset{\underset{NH_2}{|}}{C}}-CH_3\right] \longrightarrow R-\overset{\overset{O}{||}}{C}-CH_3 + NH_3$$

Eg:-

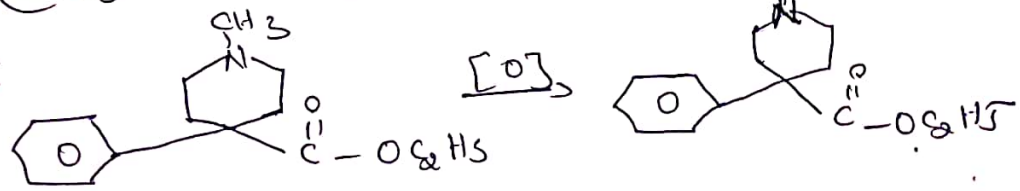
Amphetamine

phenylacetone

N- Oxide formation :-

$$(CH_3)_3N \longrightarrow [(CH_3)_3N-OH]^+ \longrightarrow (CH_3)_3N=O$$

Eg:

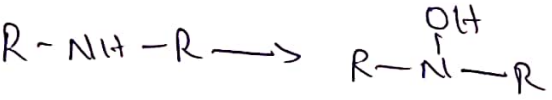


Meperidine.

Meperidine N-oxide

(18)

N-Hydroxylation



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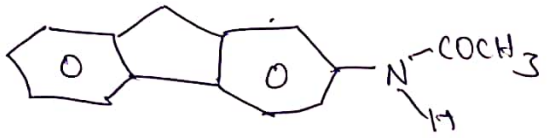
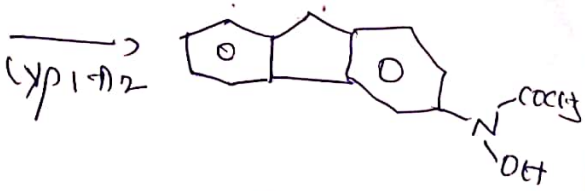


2-Acetylamino fluorene (AAF).

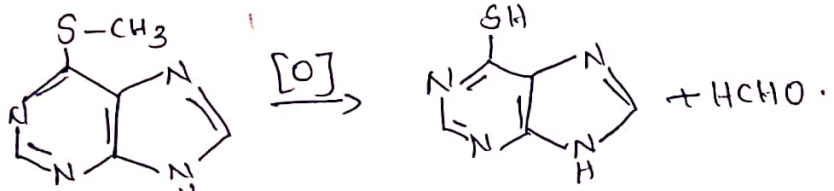


N-Hydroxy AAF

(9) Oxidation of Carbon-Sulfur System:-

Carbon-sulfur are susceptible to metabolic S-dealkylation, desulfuration and S-oxidation.

(a) S-Dealkylation:-



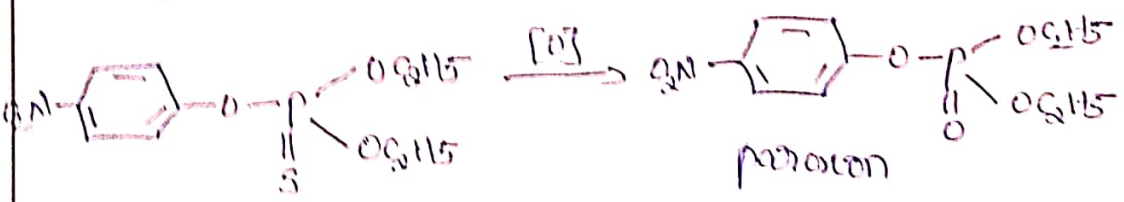
6-Mercapto

6-Methylthiopurine

6-Mercaptopurine

to

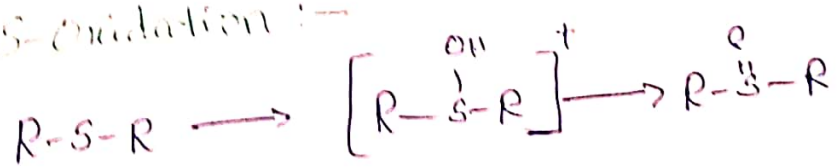
Desulfuration :-

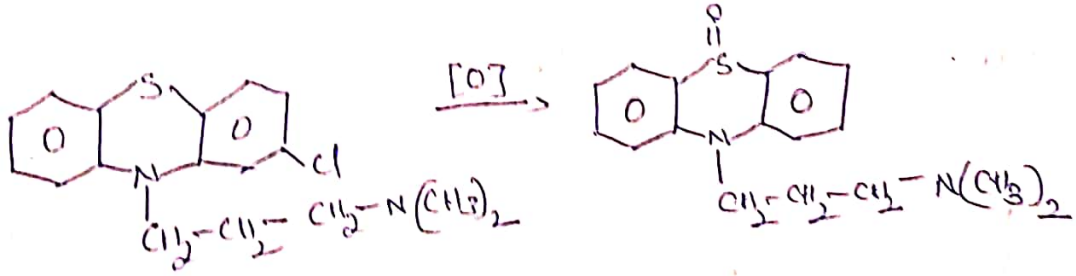


paraoxon

parathion

S-Oxidation :-



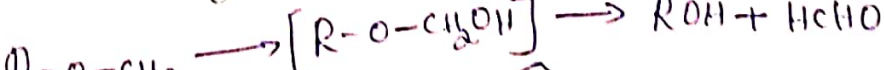


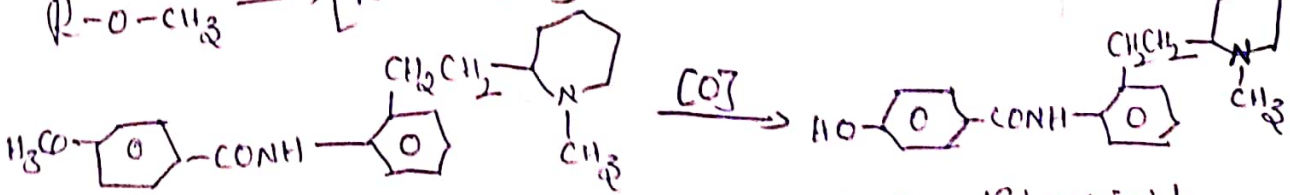
chlorpromazine

chlorpromazine sulfoxide.

Carbon-Oxygen systems :-

O-Dealkylation



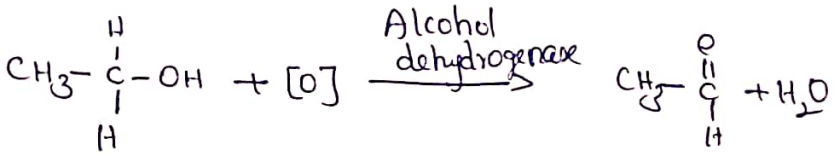


encainide.

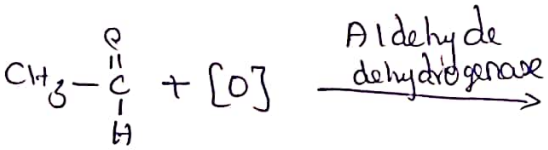
O-Demethyl encainide

(30) Oxidation of Alcohol, Carbonyl and carboxylic acid :-

(1) $RCH_2OH \xrightarrow{\text{Alcohol dehydrogenase}} RCHO \xrightarrow{\text{Aldehyde dehydrogenase}} RCOOH$



Ethanol — Ethanal

(2) 

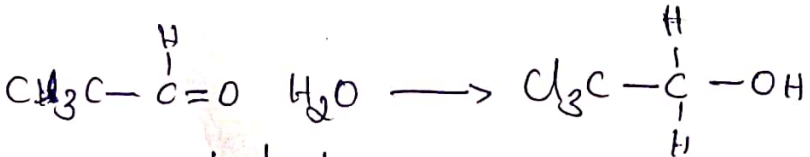
Ethanal. — Ethanoic acid.

Reduction Reactions.

Reductive reaction plays an imp. role in metabolism of many compounds containing carbonyl, nitro and azo group. Bio-reduction of carbonyl group results in alcohol where as nitro and azo group gives rise to amino group. The amino group and hydroxyl group are more susceptible to conjugation.

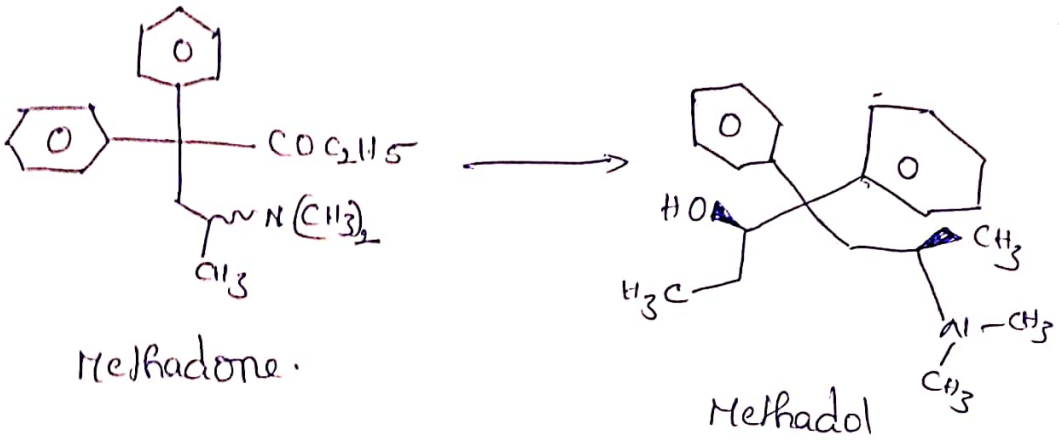
(1) Reduction of Carbonyls (aldehydes & ketones)

Aldehyde :-



chloral hydrate — Trichloroethanol.

Ketones:-

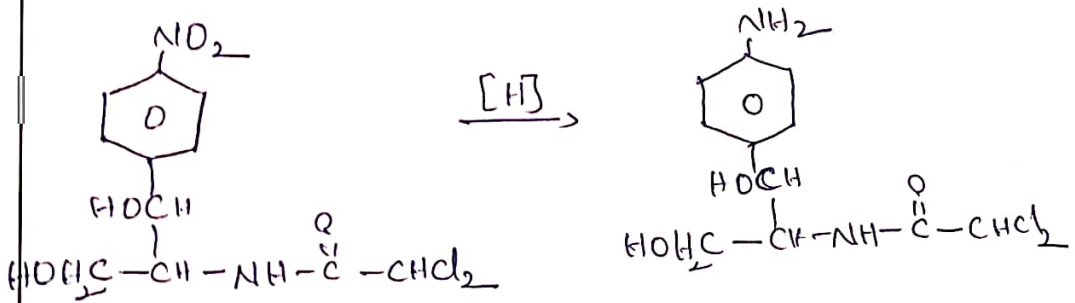


Methadone.

Methadol

Nitro reduction :-

$$RNO_2 \longrightarrow RNH_2$$

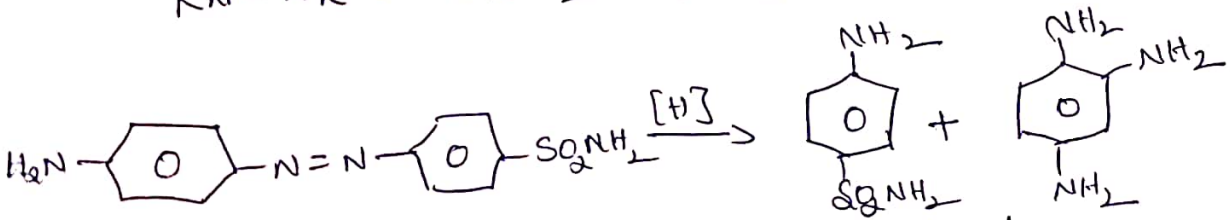


chloromphenicol

Arylamine

Azo reduction :-

$$RN=NR' \longrightarrow RNH_2 + R'NH_2$$



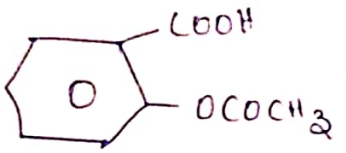
prontosil.

Sulfanilamide

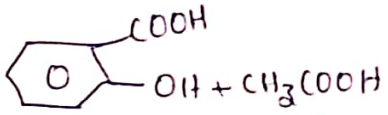
Hydrolytic reactions :—

1) Ester Hydrolysis :—

$$RCOOR' \longrightarrow RCOOH + R'OH$$



Aspirin.

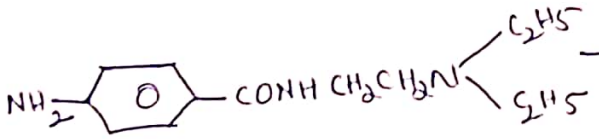


Salicylic acid

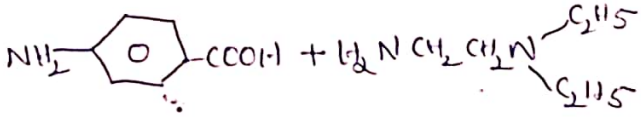
acetic acid

2) Amide hydrolysis :—

$$RCONR'R'' \longrightarrow RCOOH + HNR'R''$$



procainamide.



P-Amino benzoic acid

1-amino-2,2-(diethylamino) ethanol.

Phase-II (or) conjugation Reactions

Phase II involve transfer of suitable moiety such as glucuronic acid, sulfate, glycinate in the ~~enzyme~~ presence of enzyme transferase to drug or metabolites of phase-I having suitable functional group to form highly polar, readily excretable and pharmacologically inter conjugate

→ The phase II conjugate acetylation and methylation donot generally increase water solubility but terminate pharmacological activity.

→ phase II are real drug detoxification pathway.

- → Glucuronidation
- → Sulfate conjugation
- → Acetylation
- → glycine conjugation
- → Methylation
- → Transsulfuration
- → Glutathione conjugation
- → Mercapturic acid synthesis.

(1) Glucuronic acid Conjugation:-

→ The microsomal enzyme glucuronyl transferase conducts the donation of glucuronic acid from the endogeneously synthesized UDPGA to various substrates to form glucuronide conjugates.

eg: morphine & acetaminophen.

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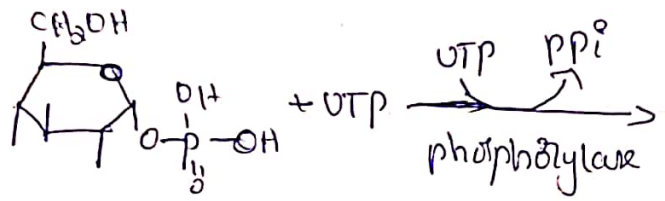
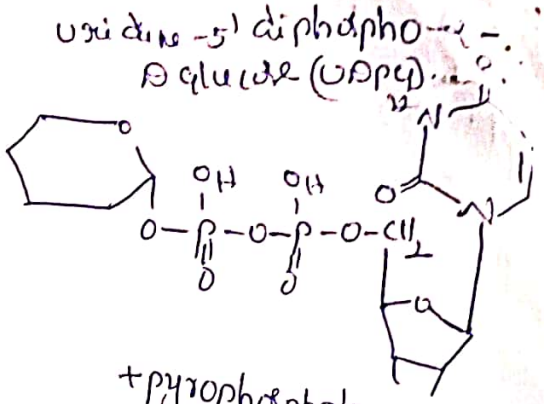
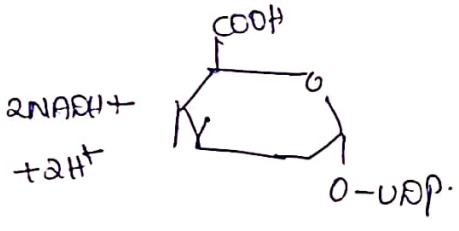
(20) Syn. of UDPGA :-



α-D glucose 1-phosphate



UDPG dehydrogenase (soluble) → 2NADH + 2NADH+2

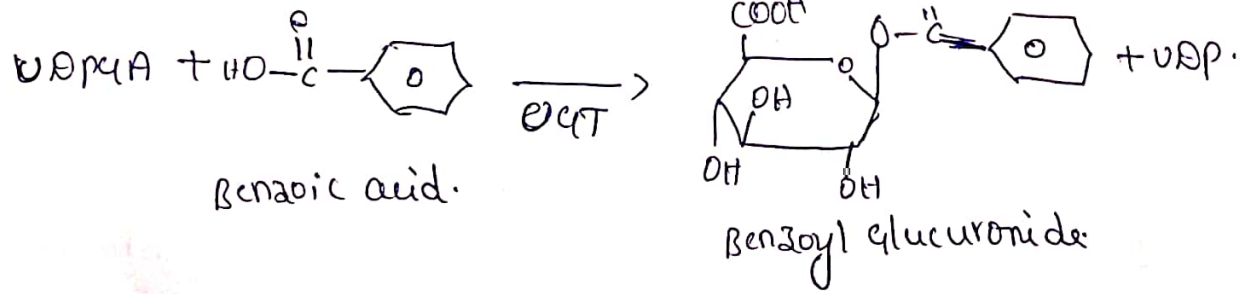


UDPG dehydrogenase

UDPG + 2NAD+ + H2O

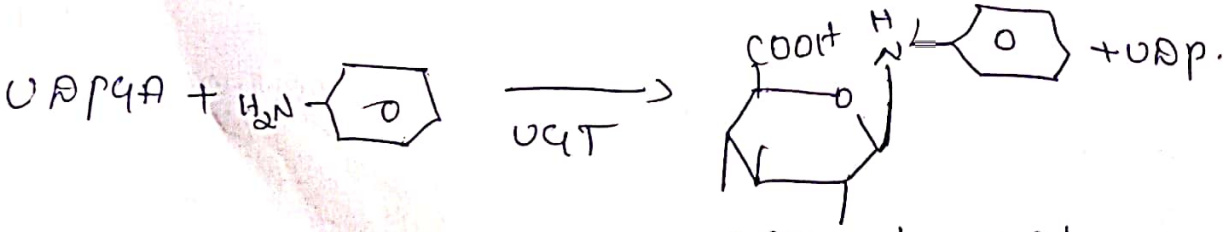
UDP-α-D glucuronic acid (UDPGA).

Glucuronidation of Benzoic acid :-



Benzoic acid.

Benzoyl glucuronide

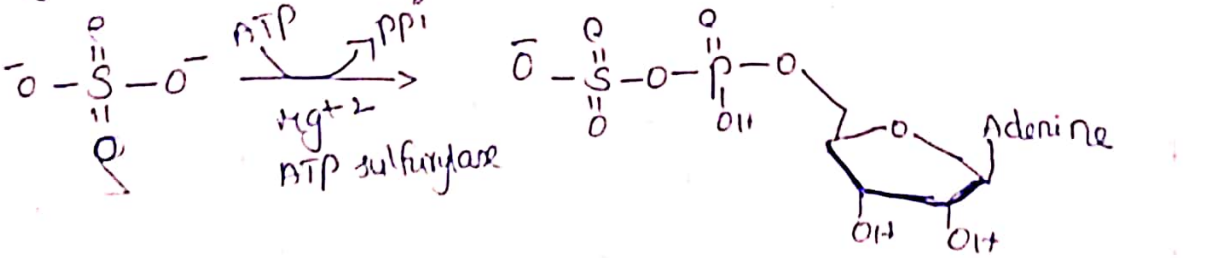


Aniline glucuronide.

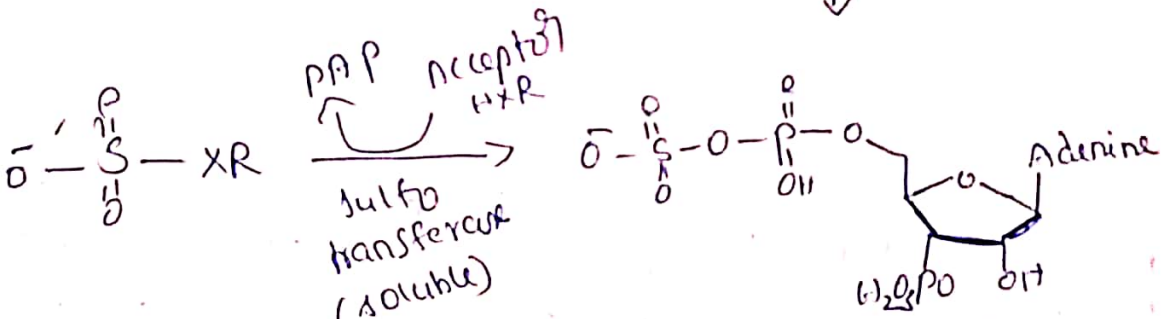
(2) Sulfate conjugation :-

- Conducted by the soluble enzyme sulfotransferase
- Endogenous donor molecule to conjugation is 3'-phosphoadenosine-5'-phosphosulfate (PAPS).
- Conjugates are ethereal in character.
- Non inducible.

Synthesis :-

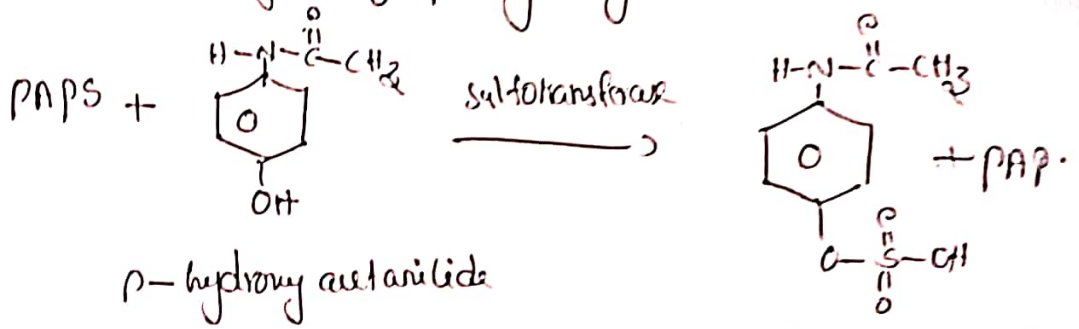


Adenosine-5'-phosphosulfate (APS).



3'-phosphoadenosine-5'-phospho-sulphate (PAPS).

① Sulfate conjugation of p-Hydroxy acetanilide



p-hydroxy acetanilide

p-Hydroxyacetanilide sulfate.

② Amino acid conjugation :- [glycine conjugation]

$$R COOH + CoA\text{-}SH \xrightarrow[ATP]{\text{Acid CoA ligase}} RCO\text{-}S\text{-}CoA$$

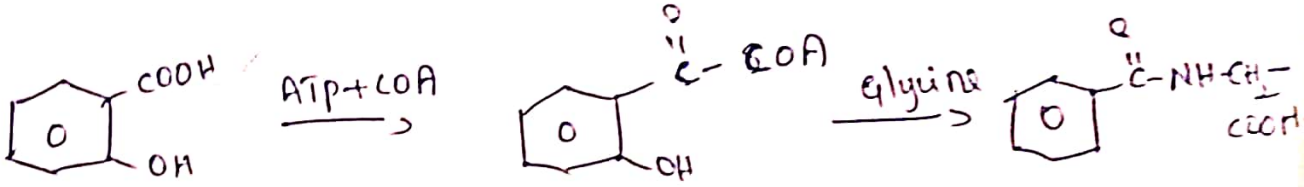
Acyl CoA

$$RCO\text{-}S\text{-}CoA + NH_2CH_2COOH \xrightarrow[\text{mitochondria}]{\text{N-Acyl transferase}} R CONH CH_2 COOH$$

glycine.

glycine conjugate

formation of glycine conjugate.



Salicylic acid.

Salicyl-CoA

Salicylic 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.

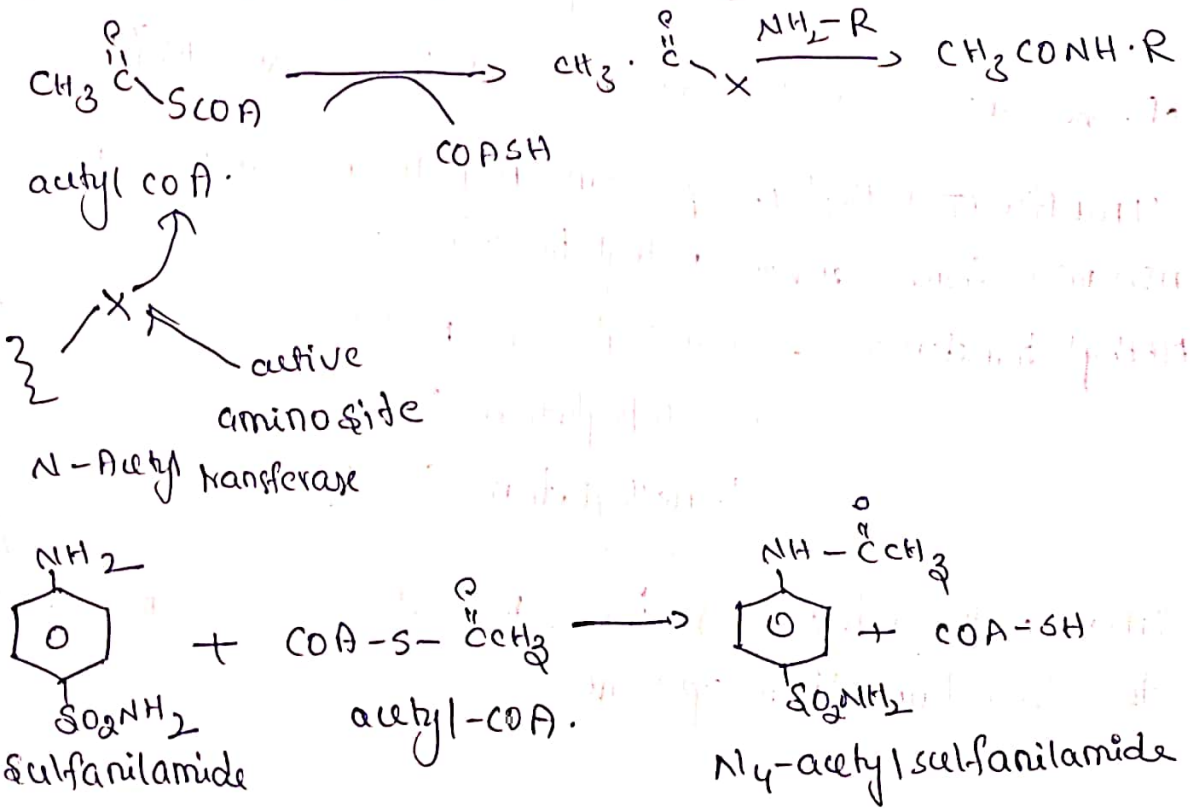
(A) Acetyl conjugation:-

→ Acetylation is an important route of metabolism for xenobiotics containing a primary amino group, including aliphatic & aromatic amine, amino acids, sulfonamides, hydrazine & hydrazides.

→ The amide formed are generally inactive, non-toxic and less water soluble. The function of acetylation may be deactivate the drug

It is a step process.

* Acetyl CoA, acetylates the active site amino acid residue of N-Acetyl transferase
* Acetyl group is transferred into the substrate amino group.



⑤ Methylation :—

→ Methylation doesn't lead to highly water soluble compounds except when methylation produces quaternary ammonium comp's.

→ Compounds undergoing methylation — phenols, amine, thiols, some nitrogen containg Heterocyclics.

→ In case of aromatic compounds containing two vicinyl hydroxy groups ie - catechol on monomethylation with the assistance of enzyme * Catechol-O-methyl transferase (COMT)

Methylation of substrate proceeds :—

* Syn. of activated methyl transferring coenzymes S-adenosyl methionine (SAM) from L-Methionine and ATP in the presence of the enzyme methionine adenosyl transferase [MAT].

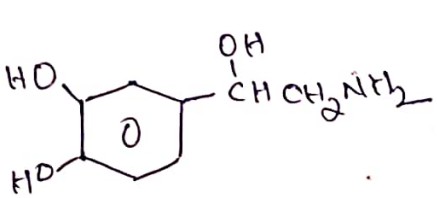
* Transfer of methyl group from SAM to the substrate in presence of non microsomal enzyme methyl transferase.

Methyl transferases — N-Methylation N-CH_3
O-Methylation O-CH_3
S-Methylation S-CH_3.

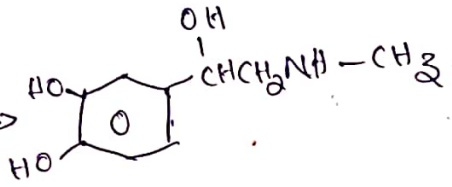
* SAM is the endogeneous donor molecule. It is demethylated to S-adenosylhomocysteine.

PNMT - phenylethanolamine -N- methyl transferase

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

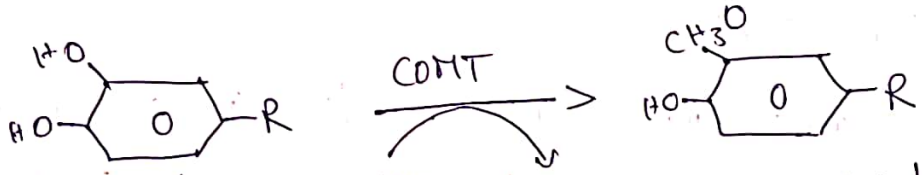


norepinephrine



epinephrine

O-Methylation of catecholamines :-

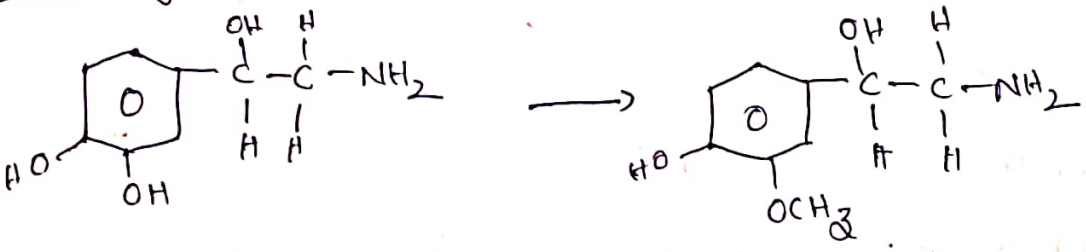


catechol

O-methylated catechol.

COMT - Catechol-O-methyl transferase

O-Methylation of Norepinephrine :-

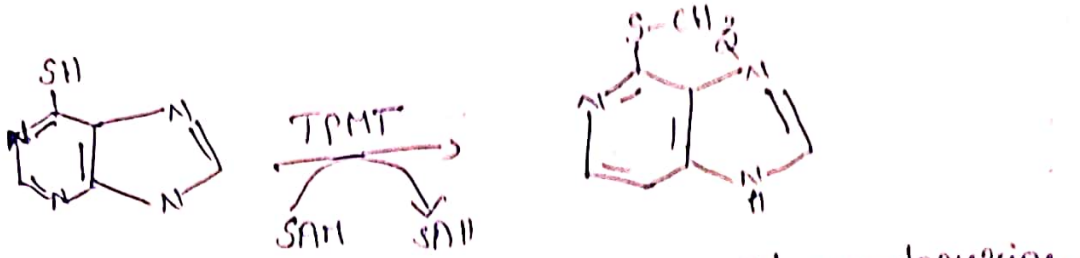


Norepinephrine.

Normetanephrine

S-methylation of 6-mercaptopurine :-

TPMT – Thiopurine methyl transferase.

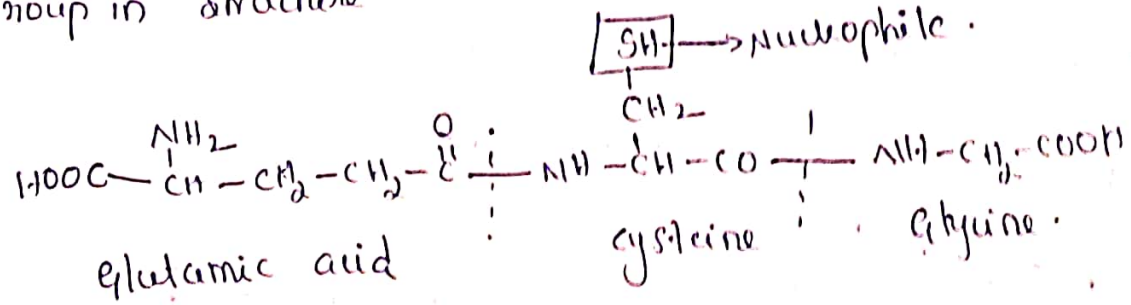


6-mercaptopurine

6-methylmercaptopurine

(6) Glutathione conjugation.

→ Glutathione (γ-glutamyl cysteinyl glycine / GSH) is a tripeptide with a strongly nucleophilic character due to the presence of a -SH (thiol) group in structure.



→ Glutathione conjugation is catalyzed by enzyme –
Glutathione – S – Transferase [GST].

GSH conjugation occurs by one of the two mechanisms:

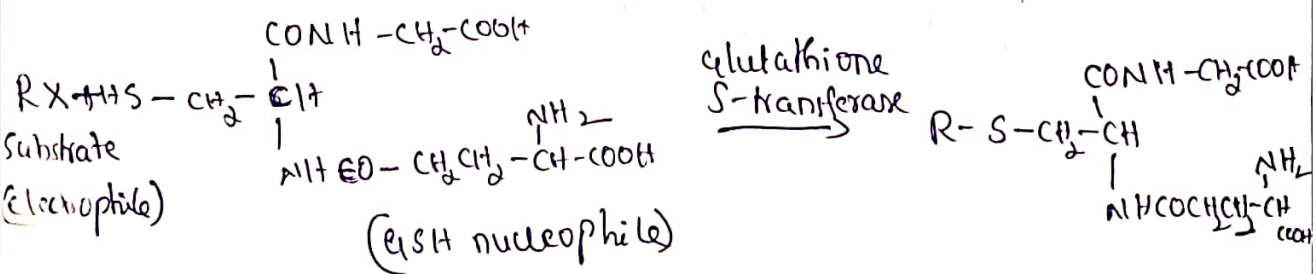
1) Nucleophilic substitution at electron deficient carbon / heteroatom such as alkyl halides, alkyl nitrates, sulfates, sulfonates, organo phosphates, epoxides, lactones etc.

$$RX + GSH \longrightarrow R\text{-}S\text{-}G + H^+ + X^-$$

2) Nucleophilic addition at the electron deficient double bond such as the α, β unsaturated carbonyl compounds eg: Ethacrynic acid.

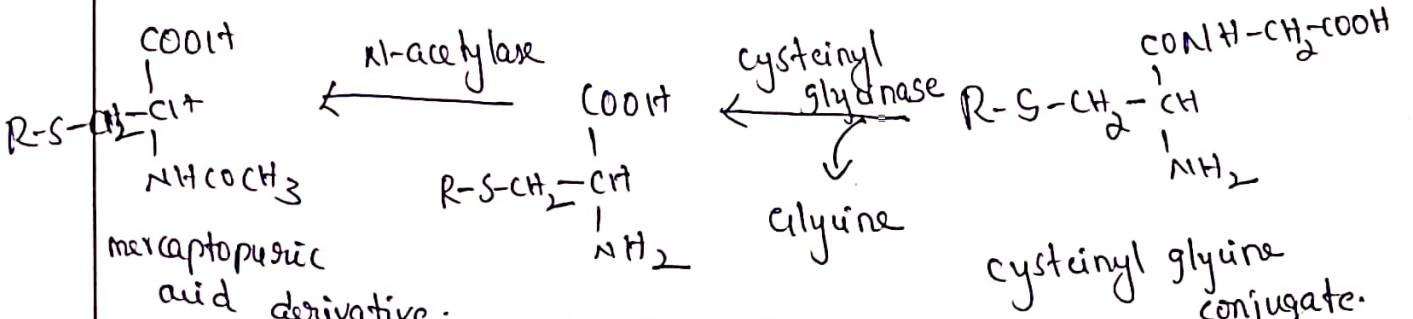
$$R\text{-}CH=CH\text{-}R + GSH \longrightarrow RCH\text{-}S\text{-}G\text{-}CH_2\text{-}R'$$

R = electron withdrawing group.



Glutathione conjugate

– Glutamine ← Glutamyl transferase



mercaptopuric acid derivative.

cysteinyl glycine conjugate.

Fate of glutathione conjugates

(7) Transsulfuration:—

$CN^- + S_2O_3^{2-} \longrightarrow CNS^- + SO_3^{2-}$

cyanide thiosulfate thiocyanate (inactive) sulfite

* mediated by mitochondrial thiosulfate sulfurtransferase (Rhodanase)

$CN^- + HS-CH_2-\overset{\ominus}{C}(=O)-COOH \longrightarrow CNS^- + CH_3-C(=O)-COOH$

cyanide β-mercaptopyruvic acide thiocyanate pyruvic acid.

* mediated by β-mercaptopyruvate sulfurtransferase.