Drug Metabolism 1

20.201

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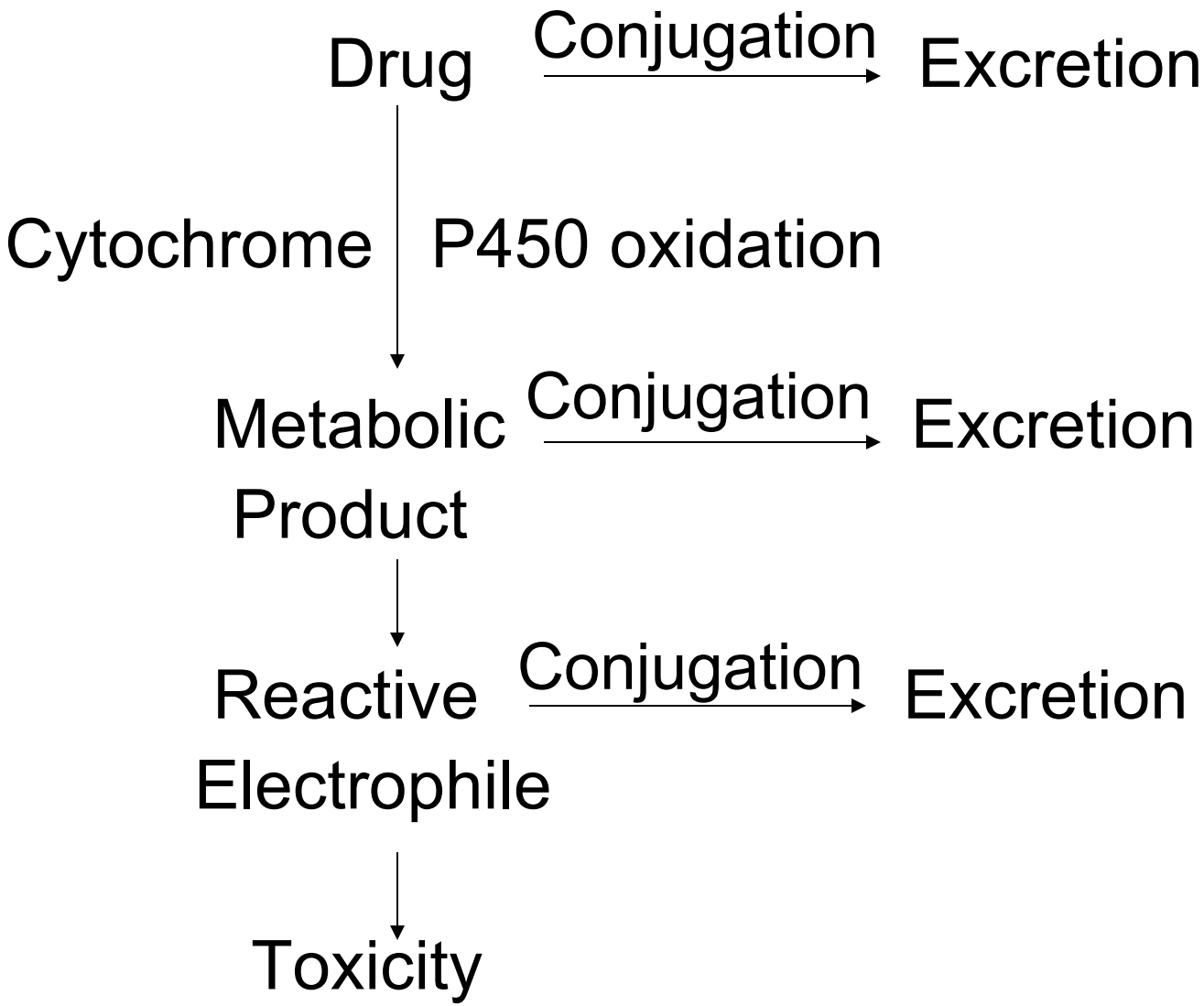
Basic Drug Metabolism



Goals

- Review the types of P450-mediated biotransformations
- Describe/postulate the mechanisms involved in these reactions
- Understand how substituents can effect both metabolic rate and metabolic pathway
- Predict both type of metabolism and where in the molecule metabolism is most likely to occur

Liver Architecture: Form follows function

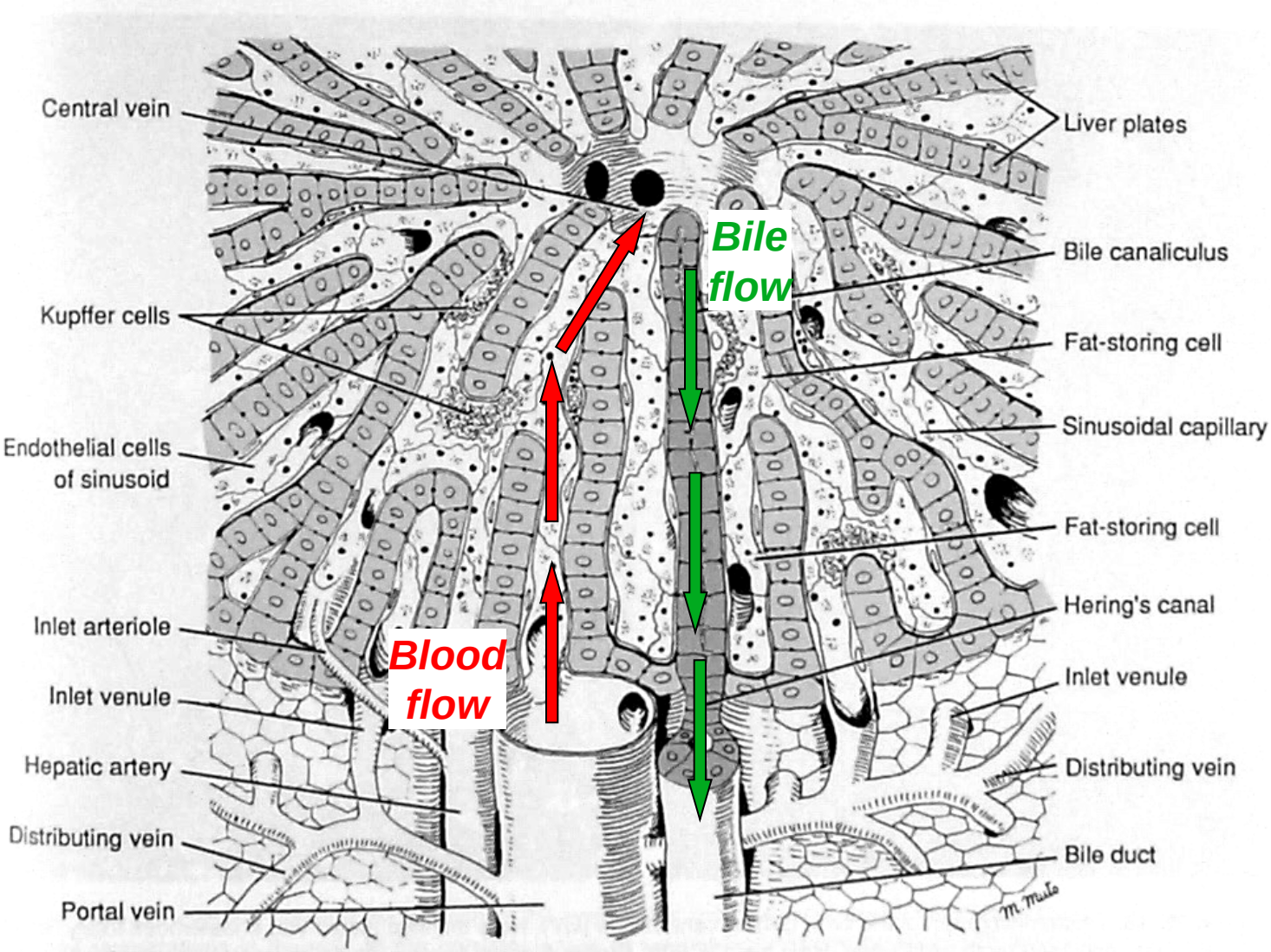


Figure 16–12. Three-dimensional aspect of the normal liver. In the upper center is the central vein; in the lower center, the portal vein. Note the bile canaliculus (darker color), liver plates (lighter color), Hering's canal, Kupffer cells, sinusoid, fat-storing cell, and sinusoid endothelial cells. (Courtesy of M Muto.)

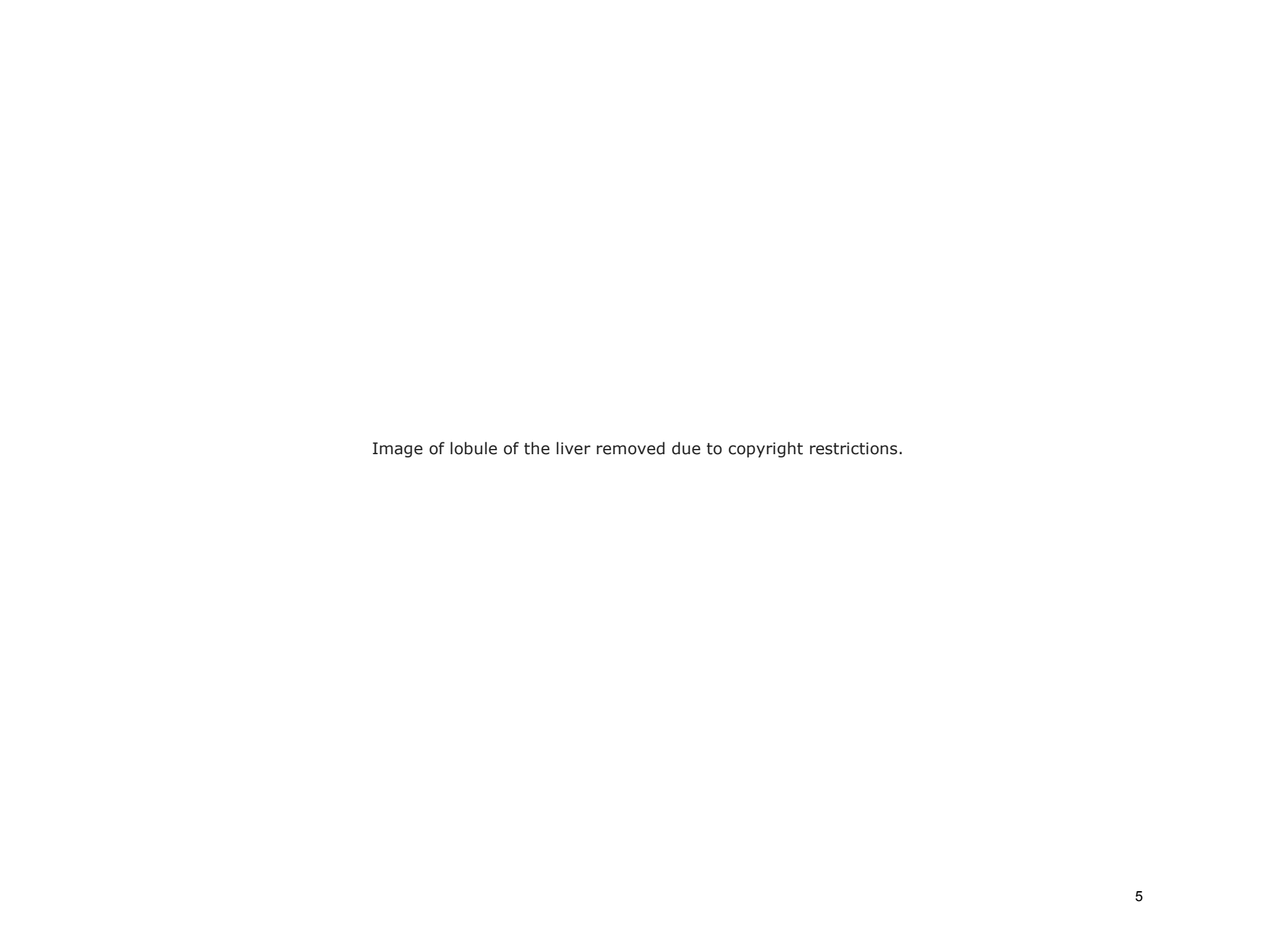


Image of lobule of the liver removed due to copyright restrictions.


Schematic image of liver sinusoidal cells removed due to copyright restrictions.

Cytochromes P450

•P450s are a family of closely related enzymes *(isoforms)* that catalyze the same reaction(s) on different substrates

•termed mixed function oxidases because they transfer one atom of molecular oxygen (O_2) to the substrate and the other undergoes a two electron reduction and is converted to water

•functional enzyme consists of a membrane bound hemoprotein and a soluble flavoenzyme, P450 reductase, which transfers electrons to P450

•catalyze the oxidation, reduction or hydrolysis of both endogenous and exogenous substrates

•reactions are believed to proceed via free radical mechanisms *(HAT vs SET)*

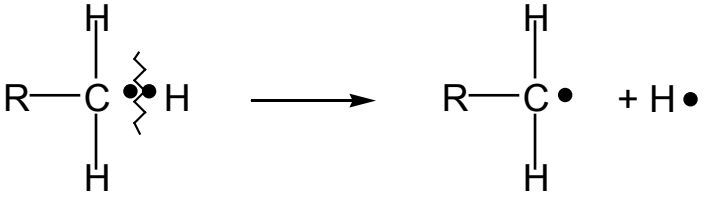
•levels of these hemoproteins can be influenced by many chemicals and the enzymes in turn are capable of metabolizing many compounds

Free Radicals in Enzyme Reactions

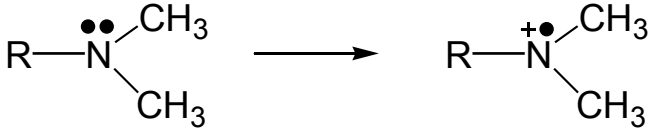
What is a free radical?

A free radical refers to any molecule that has an odd number of electrons.

Free radicals generated from the cleavage of covalent bonds (e.g., homolytic bond breaking of C-H bonds) are symbolized with a single dot which represents the unpaired electron.



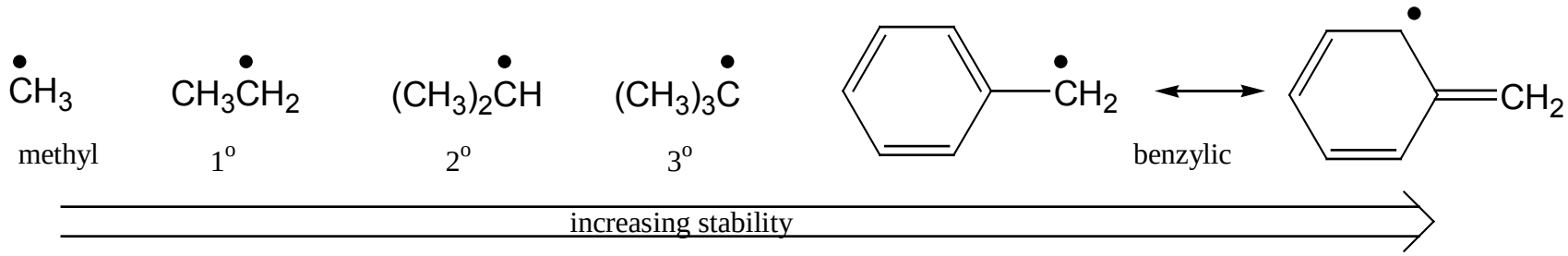
Abstraction of a single electron on either N, P or S results in the formation of charged radical and is referred to as a cation radical (•+).



Factors that influence where a radical will form in a molecule upon reaction with Cytochrome P450

1. Electronics of the substrate which influence the “ease” of H atom abstraction or electron transfer (i.e., electron withdrawing vs electron donating properties)

2. Stabilization of the radical intermediate via resonance or inductive effects (allyl ~ benzyl>3°> 2°>1°)



3. Steric considerations

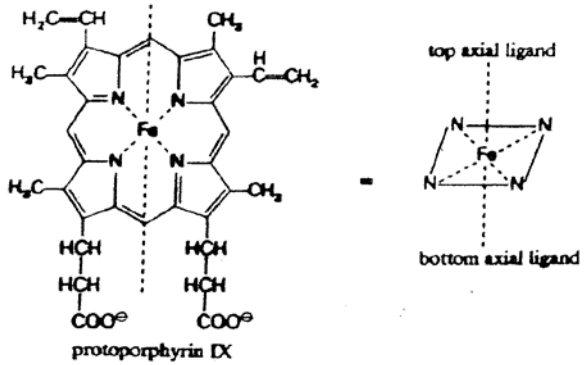
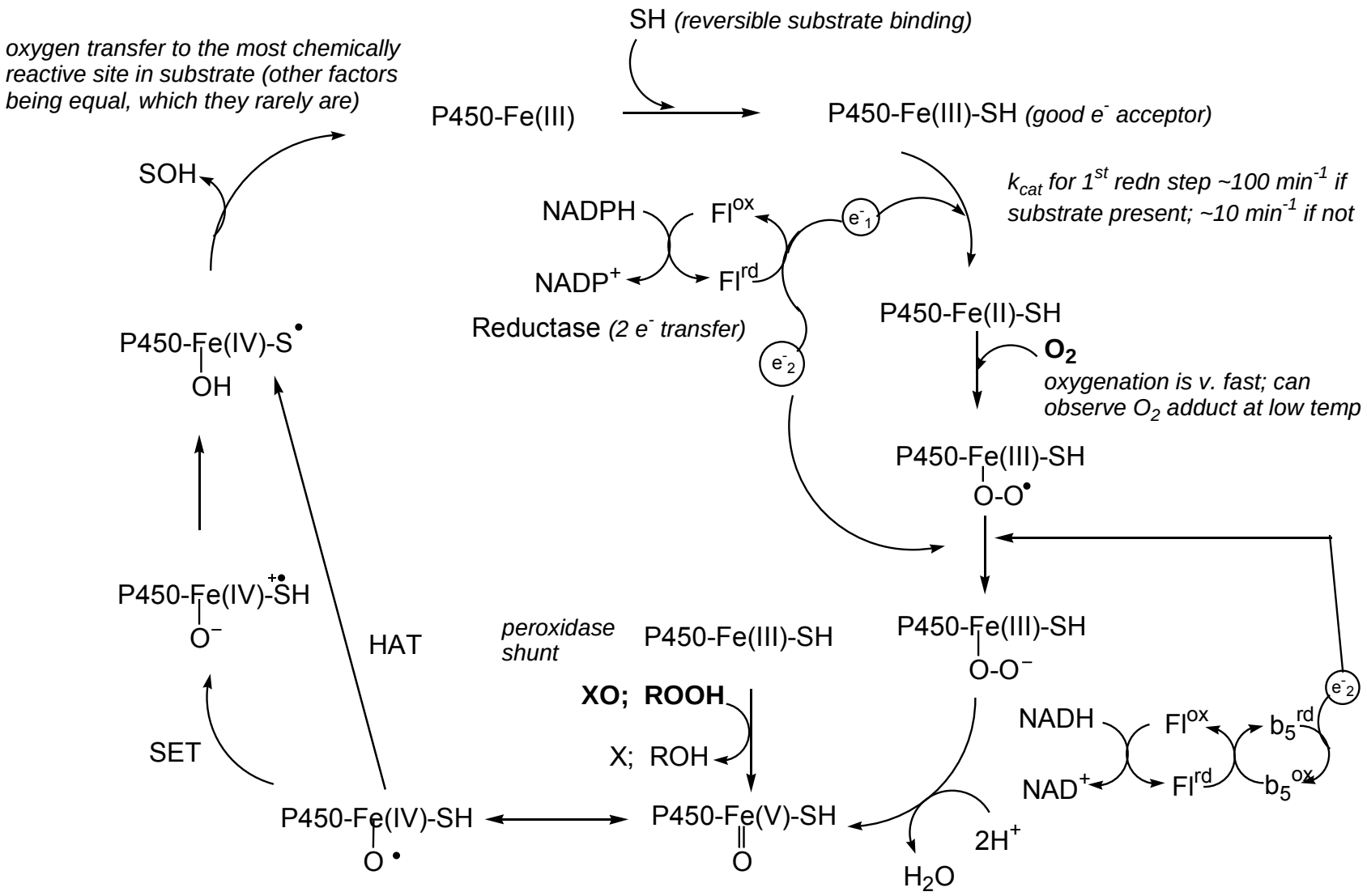


Figure of absorbance vs wavelength (nm) removed due to copyright restrictions.

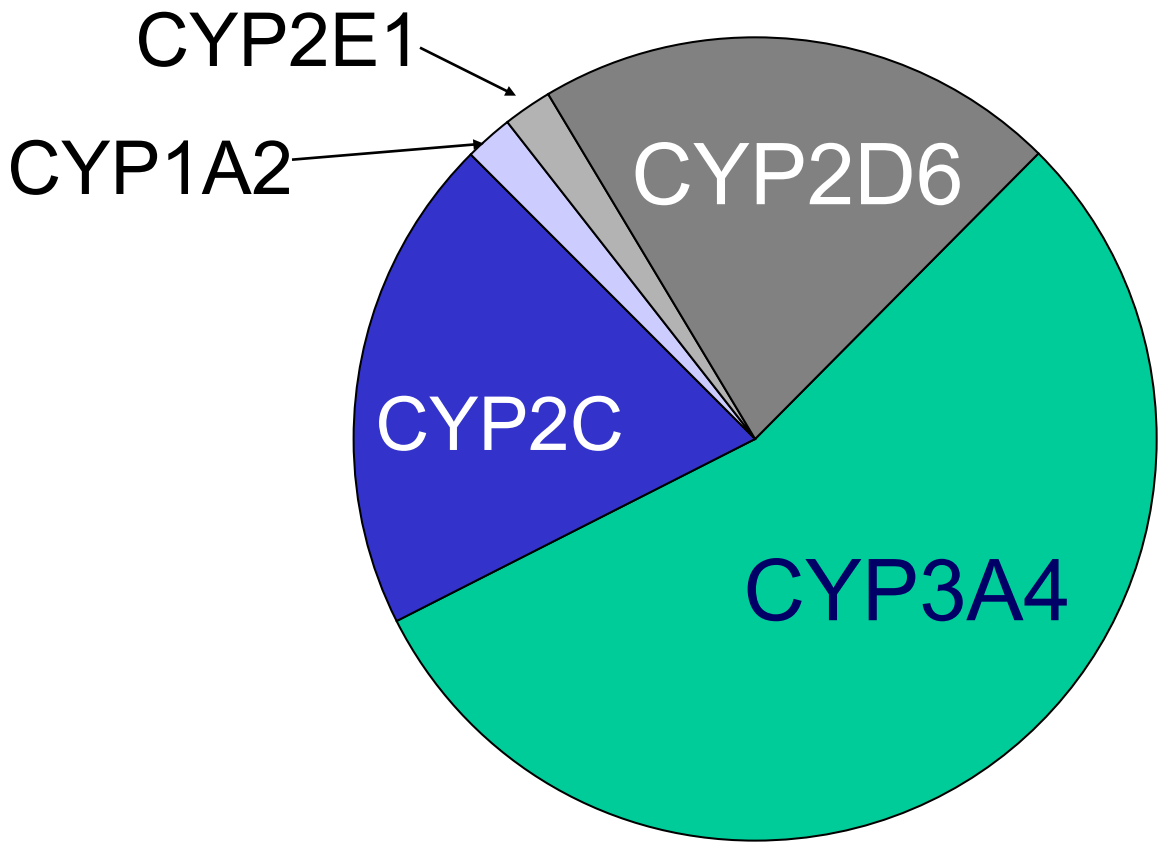

Image of horseradish peroxidase, chloroperoxidase, and catalase removed due to copyright restrictions.

Figure of cooxidation of xenobiotics (X) during the conversion of arachidonic acid to PGH2 by prostaglandin H synthase removed due to copyright restrictions.

The P450 Cycle



Major Human CYPs Responsible for the Metabolism of Current Drugs



More than 90% of the therapeutics on the market are metabolized by these P450s. Therefore, interaction with these CYPs could be clinically relevant!

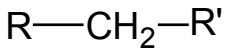
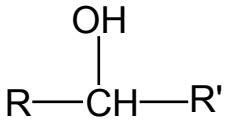
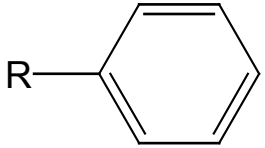
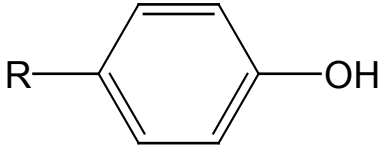
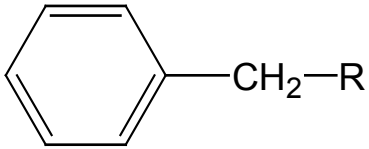
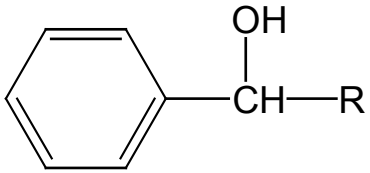
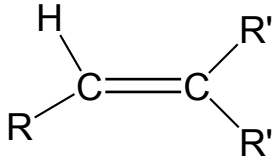
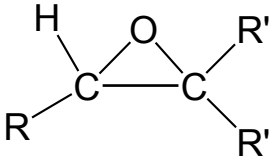
General Properties of Human P450 Substrates

(Lewis, Biochem Pharmacol, 60, 291-306, 2000)

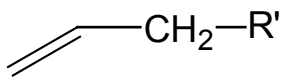
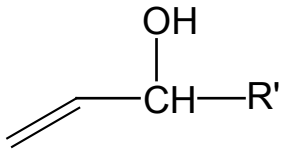
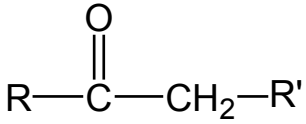
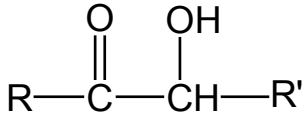
- 1A2: planar, moderately basic, medium volume
- 2A6: non-planar, medium volume
- 2C9: weakly acidic, lipophilic, 1 or 2 H-bond donor/acceptor at 5-8 A from the site of metabolism
- 2C19: neutral or weakly basic, 2-3 H-bond donor/acceptors at 4.5 A apart and 5-8 A from the site of metabolism
- 2D6: basic, relatively hydrophilic, a H-bond donor/acceptor at 5-7 A from site of metabolism
- 2E1: low volume, neutral, hydrophilic
- 3A: high volume, lipophilic, structurally diverse, 1-2 H-bond donors/acceptors at 5.5-7.5 A and 8-10 A from the site of metabolism

Classes of Substrates for Cytochrome P450

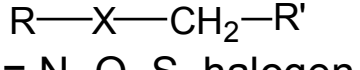
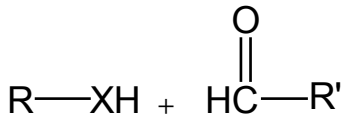
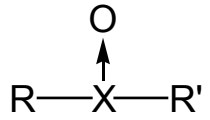
I. Oxidation of C-H Centers

Functional Group	Product
 aliphatic carbon	 alcohol
 aromatic carbon	 aromatic hydroxylation
 benzylic carbon	 benzylic hydroxylation
 alkene	 epoxide

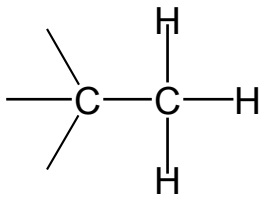
I. Oxidation of C-H Centers cont'd

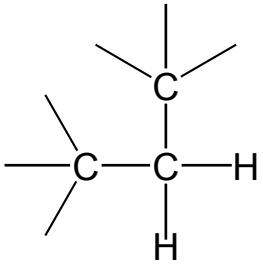
Functional Group	Product
 allylic carbon	 allylic alcohol
 acyl carbon	 acyl alcohol

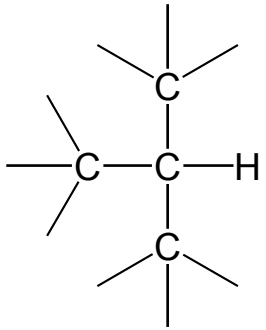
II. Oxidation of Heteroatoms (dealkylation, oxygenation)

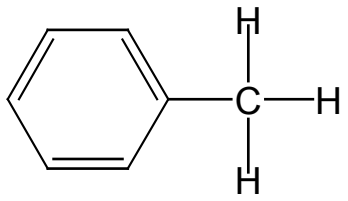
Functional Group	Product
 X = N, O, S, halogen	 dealkylated product
 X = NR", S	 N or S oxide

Hydroxylation of Aliphatic Carbon Centers

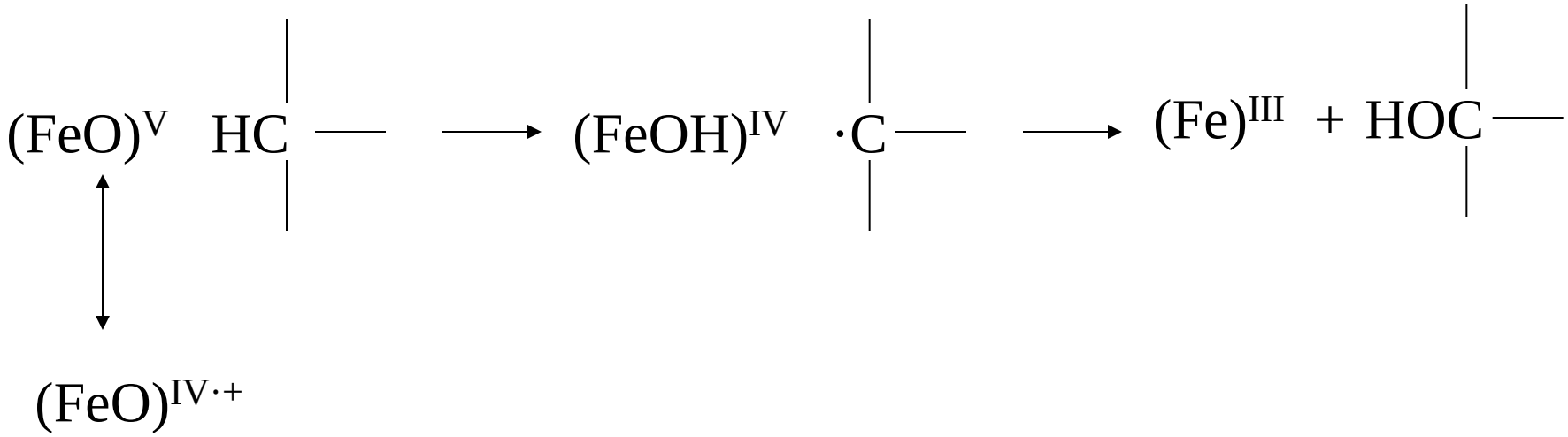








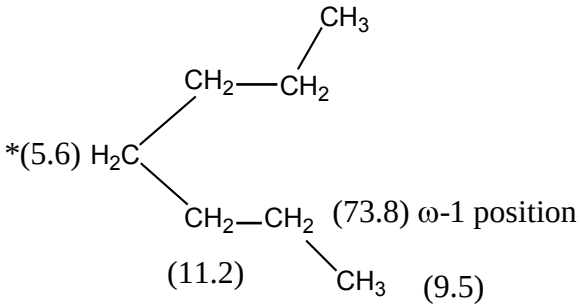
Mechanism of Aliphatic Hydroxylation (HAT)

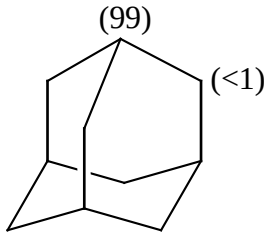


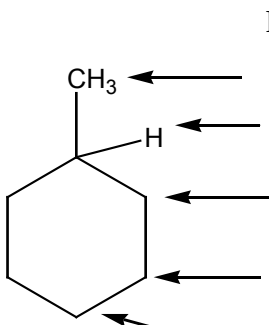
- stepwise process-loss of stereochemistry
- hydrogen atom abstraction and carbon radical formation
- collapse of carbon radical perferric hydroxide radical pair (radical recombination)
- large isotope effect (k_H/k_D >7) due to C-H bond breaking

Characteristics of Aliphatic Hydroxylation via P450

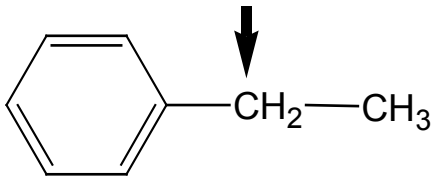
I. Regioselectivity







	P450	CF_3CO_3H
CH_3	(1)	(0.2)
H	(25)	(89)
	(8)	(3)
	(43)	(3)
	(23)	(4)

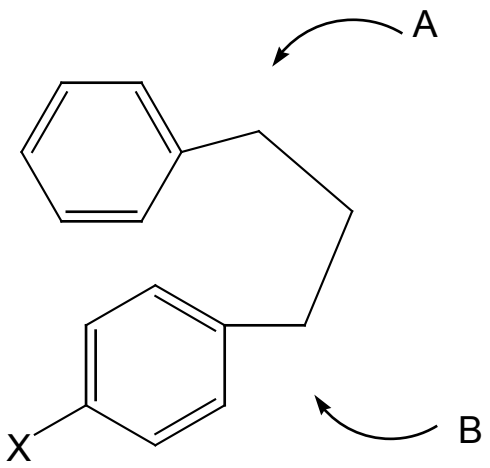


Relative reactivity order for H• abstraction of C-H groups

allyl ~ benzyl > 3° > 2° > 1°

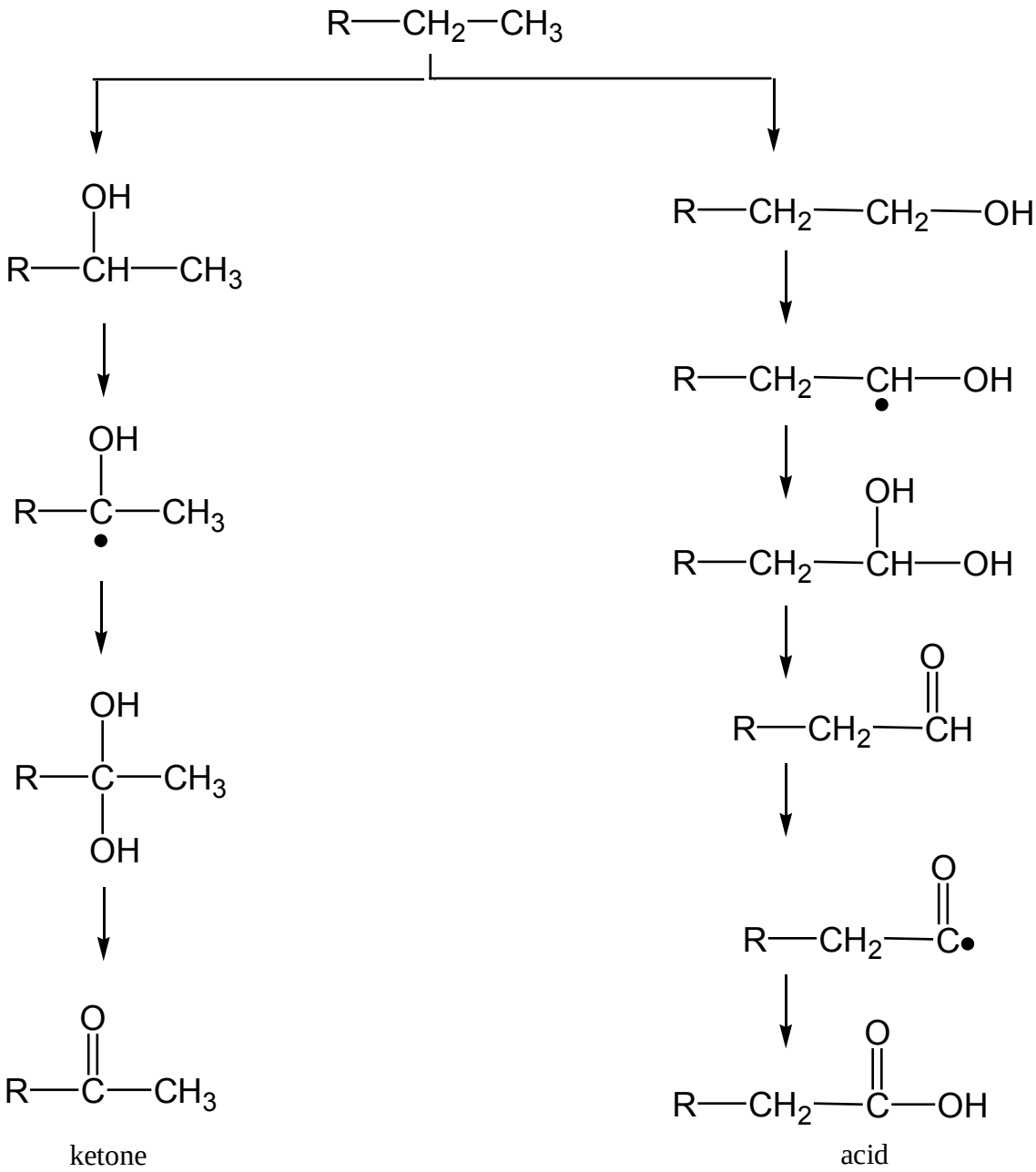
**numbers in parenthesis indicate the percent of hydroxylation occurring that site*

II. Substituent effects (electron donation vs electron withdrawal)

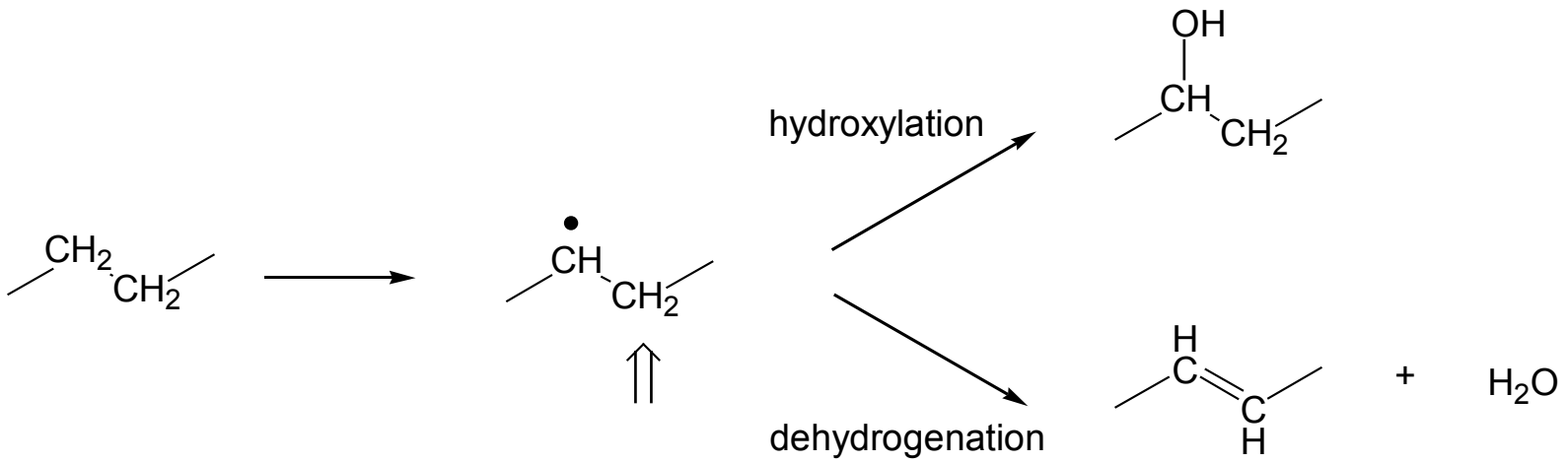


X	A-OH	B-OH
CH_3	50	50
F	70	30
CF_3	95	5

III. Subsequent Metabolism of Hydroxylated Hydrocarbons

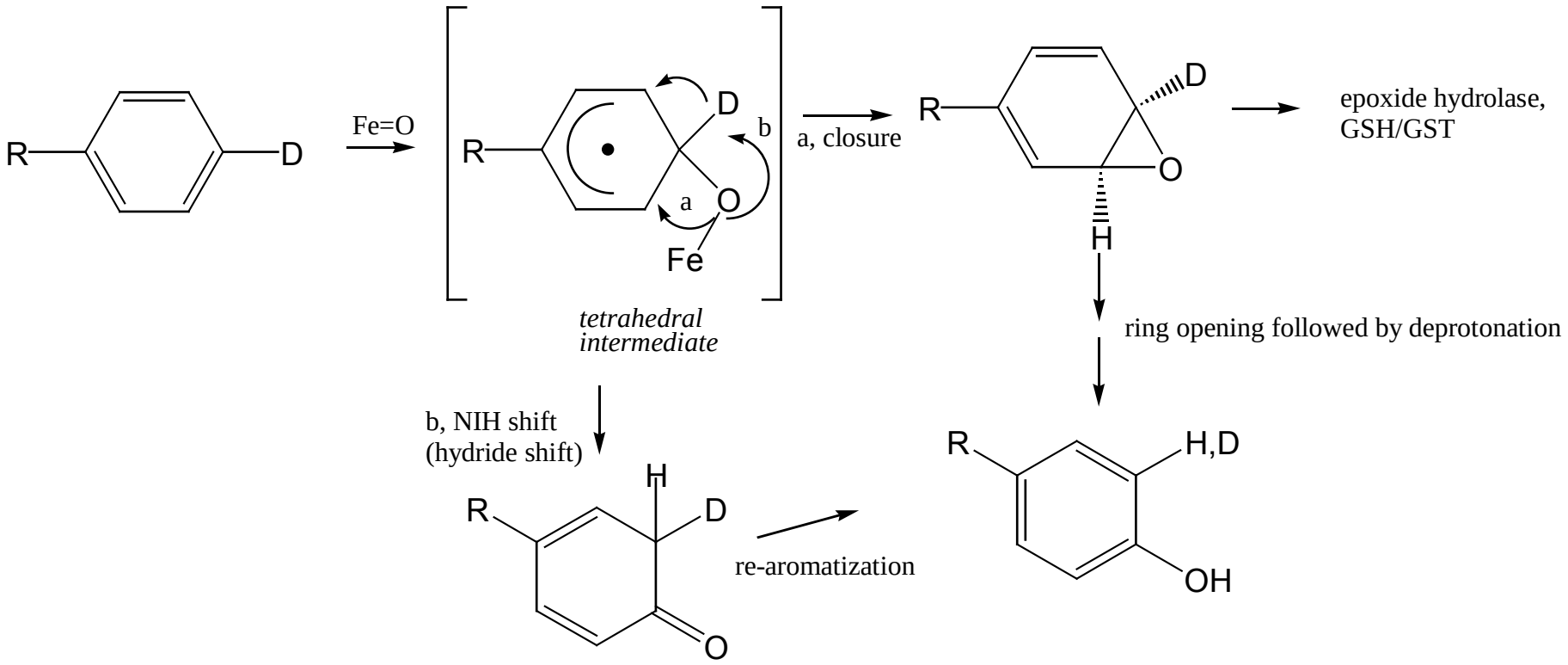


Alkane Biotransformtion Pathways



- hydrogen atom abstraction and carbon radical formation
- oxygen rebound for hydroxylation
- removal of β-hydrogen for dehydrogenation

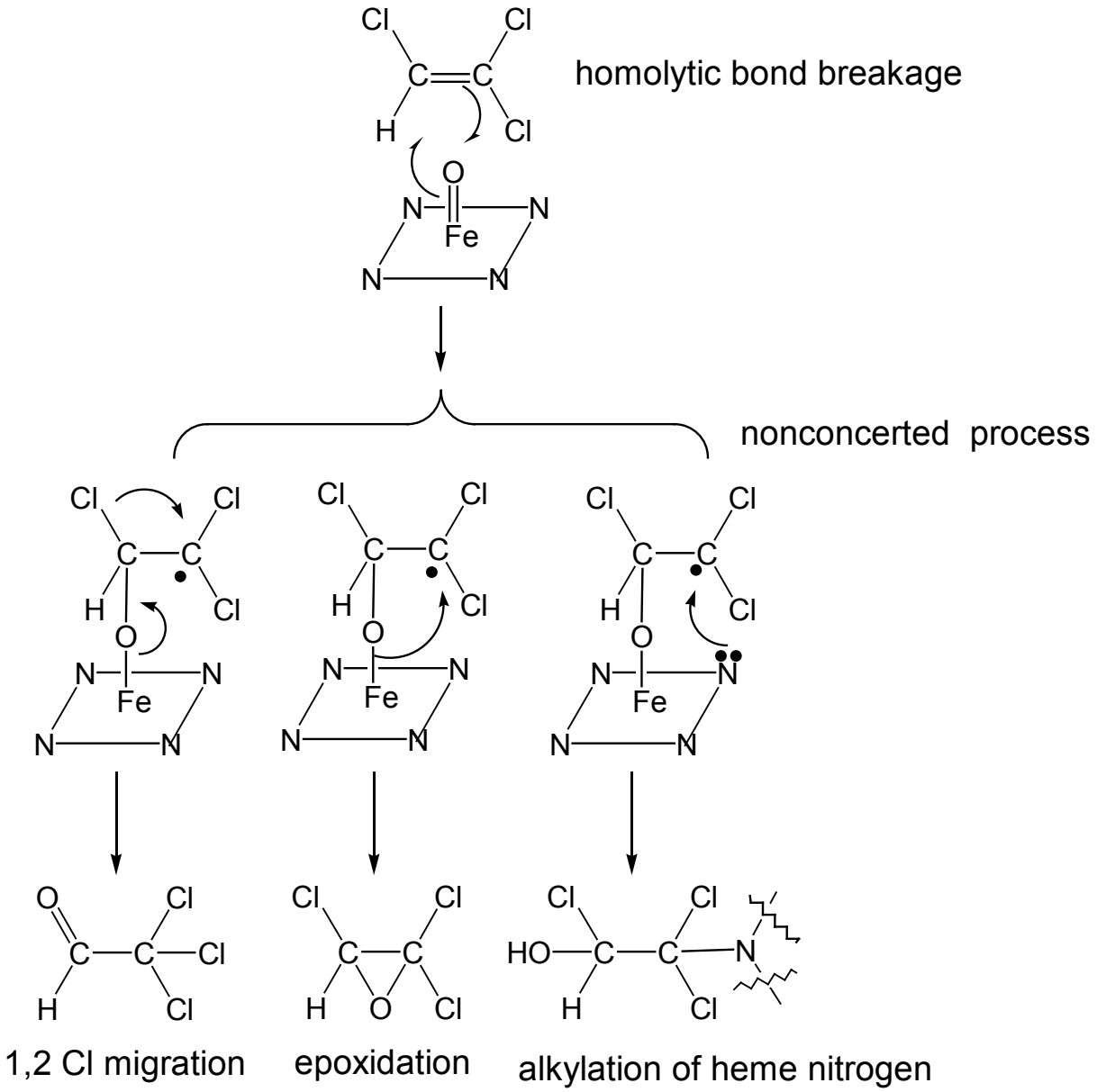
Aromatic Hydroxylation



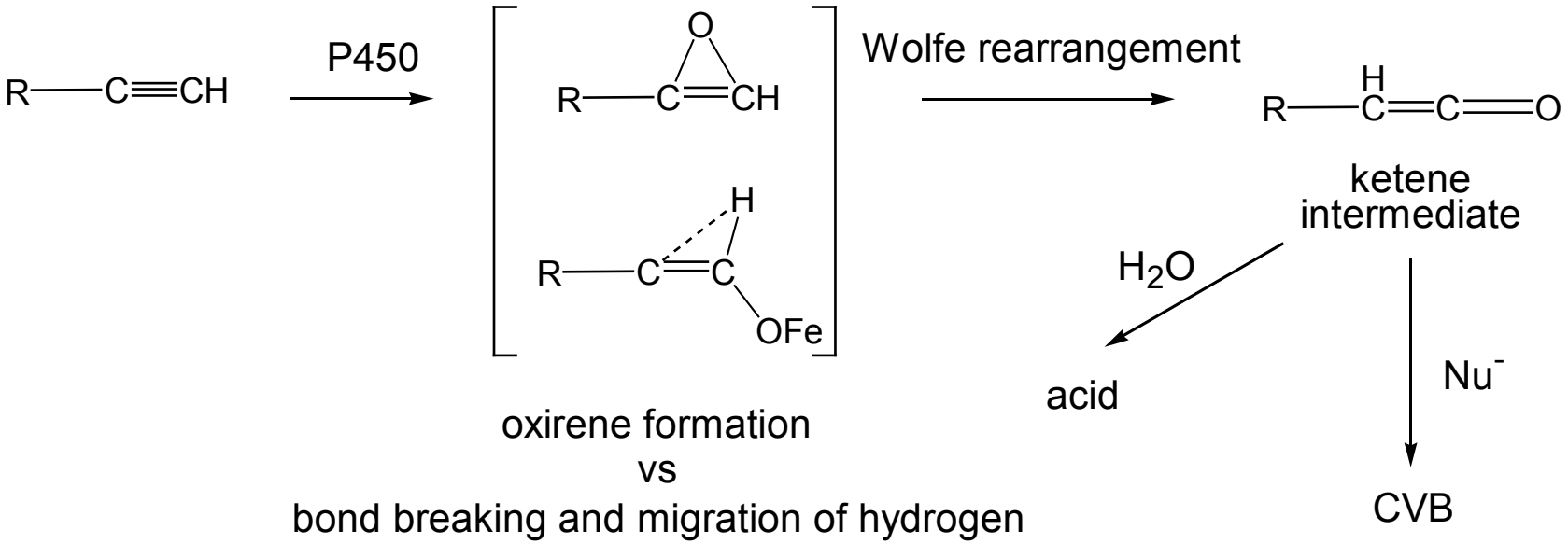
overall rates are proportional to e^- density

- *when R is electron donating: para > ortho >meta hydroxylation*
- *when R is strongly electron withdrawing little to no aromatic hydroxylation occurs*

Oxidation of Alkenes

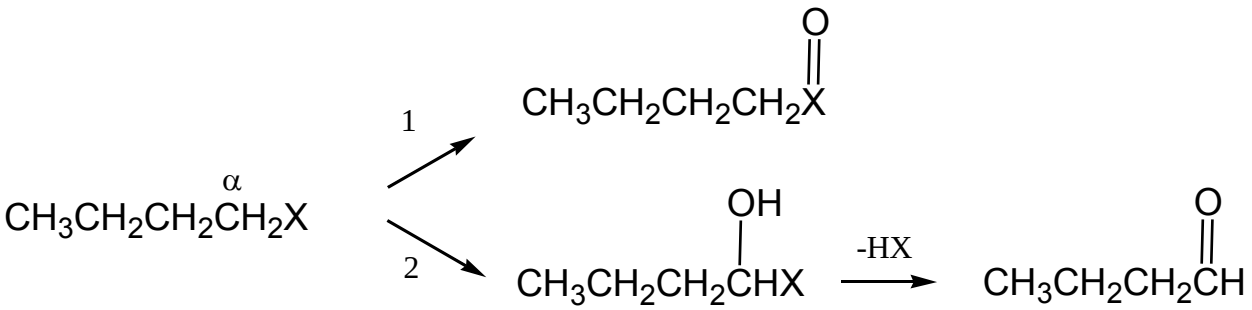


Oxidation of Acetylenes



Heteroatom Dealkylation and Oxygenation Reactions

These processes are related conceptually by examination of the site in the heteroatom-containing substrate to which oxygen is transferred. In heteroatom oxygenation[1], oxidation occurs at the heteroatom, whereas in dealkylation reactions[2], oxygen is transferred to the carbon adjacent (α) to the heteroatom.



Heteroatom Oxygenation vs Dealkylation

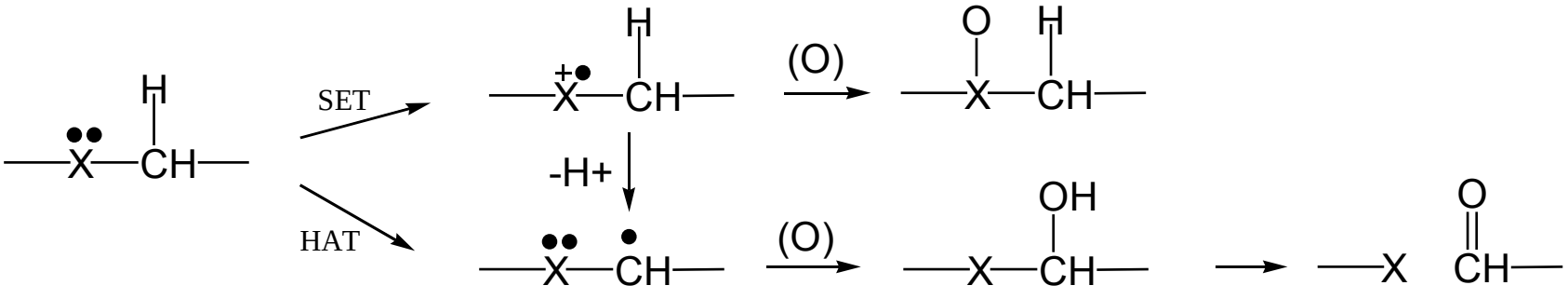
• Heteroatom oxygenation occurs via an initial abstraction of an electron from the heteroatom (SET) resulting in the formation of a radical cation intermediate

• Dealkylations are generally thought to undergo an initial H-atom abstraction (HAT) resulting in the formation of a neutral carbon radical intermediate

• For carbon-oxygen systems (R-OCH_3), heteroatom oxygenation is not possible due to the high ionization potential of oxygen

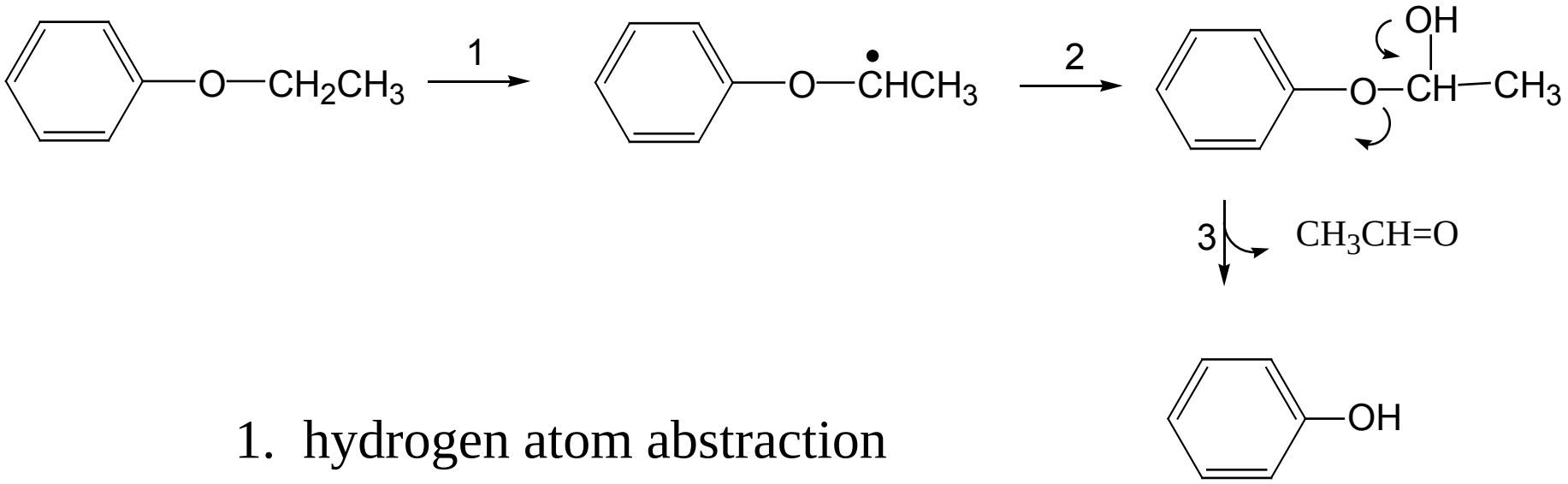
• For carbon-nitrogen systems, *N*-dealkylation is favored when an α H^+ is present, whereas, *N*-oxygenation occurs in the absence of α H^+ protons or if the radical cation intermediate is stabilized via a nearby EDG

• For carbon-sulfur and carbon-phosphorous systems, heteroatom oxygenation is favored due to formation of a stable radical cation



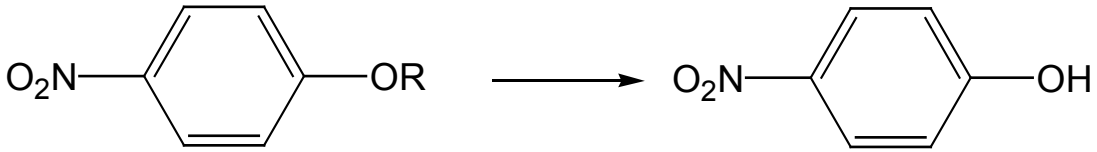
Oxidation of Carbon-Oxygen Systems

O-Dealkylation (HAT)



1. hydrogen atom abstraction
2. oxygen rebound
3. disproportionation

Substituent Effects on the Rate of *O*-Dealkylation by P450

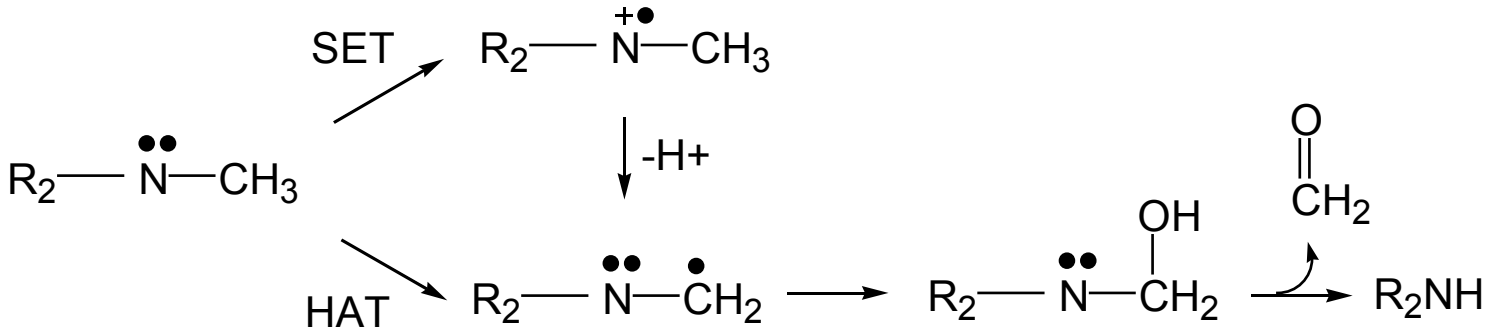


p-Nitro Phenol Formation

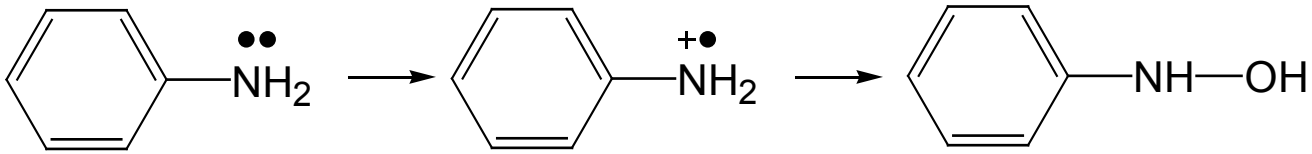
R	K_M	V_{MAX}	V/K
CH_3	16.7	143	0.68
Ethyl	0.68	72	1.05
Propyl	1.2	38	3.16
Iso Propyl	0.64	34	5.13
Butyl	0.42	8	1.9

Oxidation of Carbon-Nitrogen Systems

1. *N*-Dealkylation (αHs, HAT or SET)

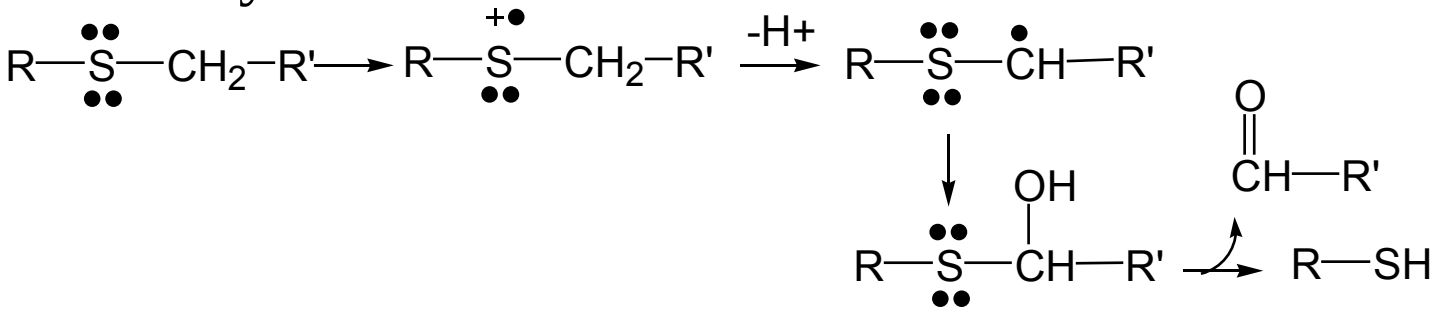


2. *N*-Oxidation (No αHs, SET)

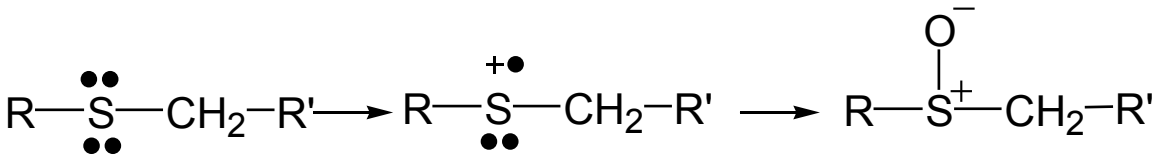


Oxidation of Carbon-Sulfur Systems

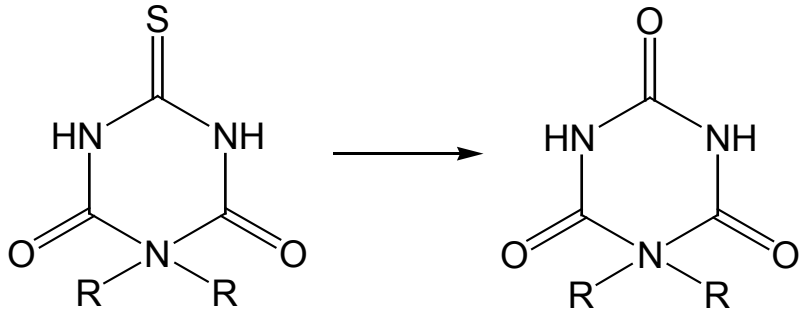
1. S-Dealkylation



2. S-Oxidation



3. Desulfuration (suggest a mechanism)



20.201 Mechanisms of Drug Actions

Fall 2013