MECHANISTIC PATHWAYS TO PROCESS-INDUCED TOXICANTS IN MEAT

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Abstract - This review surveys various mechanistic routes to the thermally generated toxicants in meat described in the literature, focusing mainly on heterocyclic aromatic amines (HAA) and polycyclic aromatic hydrocarbons (PAH). Two types of precursors were identified for their formation; one pre-existing in meat such as specific amino acids (tryptophan, phenylalanine, etc.) or creatine/creatinine and second those that are generated during thermal processing through either Maillard reaction such as pyrazines or pyridines, Strecker aldehydes such as phenylacetaldehyde, formaldehyde and acetaldehyde or lipid oxidation/thermal degradation products such as alkenes, dienes and short chain aldehdyes of which some are similar to those generated through the Maillard reaction.

Key Words - Heterocyclic aromatic amines (HAA), polycyclic aromatic hydrocarbons (PAH), PhIP, mechanisms of formation.

I- INTRODUCTION

Consumption of red meat (beef, pork, and lamb) is among the top ten risk factors associated with carcinogenesis (Wiseman, 2008). One of the underlying mechanisms proposed for the cancer-causing effect of red meat is the formation of chemical carcinogens and mutagens during various meat processing operations such as curing, smoking, fermentation and thermal treatments. Each of these operations can generate specific type of chemical toxicants (Behesnilian et al, 2014) such as N-nitroso compounds are formed during curing of meat with nitrites, heterocyclic aromatic amines (HAA) are generated during frying or char-grilling from the interaction of creatinine with the Maillard reaction (Hellwig and Henle, 2014) and/or lipid oxidation products (Zamaro and Hidalgo, 2015), biogenic amines are mainly generated during fermentation of meat products due to the presence of specific amino acids and decarboxylase positive microorganisms and lastly polycyclic aromatic hydrocarbons (PAH) are formed during incomplete combustion of wood and smoke generated from heated lipids and oils (Chen and Chen, 2001) due to the chemical interaction of pyrolysis products of fats and carbohydrates (Table 1).

Although detailed understanding of the mechanism of formation of process-induced toxicants can help to formulate appropriate mitigation strategies (Perez and Yaylayan, 2008), however, only few studies are reported on the mechanistic pathways and most studies so far are directed towards analytical techniques (Yan et. al., 2014; Penga and Turesky, 2014) or towards attempts to reduce their formation through the addition of various spices and antioxidants (Viegas et. al., 2014) in addition to understanding their kinetics and modeling of their formation (Kondjoyan et al., 2014).

This review will mainly focus on carcinogenic structures generated at high temperatures of processing of meat such as heterocyclic aromatic amines (HAA) and polycyclic aromatic hydrocarbons (PAH) specifically 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) which is targeted by most of the recent mechanistic studies.

II- PROPOSED PATHWAYS TO HETEROCYCLIC AROMATIC AMINES (HAA)

Heterocyclic aromatic amines can be classified into two categories (see Figure 1); one almost invariably incorporates a creatine moiety and is known as aminoimidazoazarenes and the second grouping requires a higher temperature of generation from pyrolysis of various amino acids and is known as pyrolytic heterocyclic aromatic amines. Aminoimidazoazarenes can be grouped further into several categories, such as imidazopyridines, imidazoqiuinolines and imidazoquinoxalines. On the other hand, pyrolytic heterocyclic aromatic amines can be classified into various carboline derivatives such as α-, β-, γ- and other derivatives.

2.1 PhIP

Most efforts to understand the mechanism of formation of process-induced toxicants have been directed towards the investigation of the mechanism of formation of PhIP (see Figure 2) a member of aminoimidazopyridine group as shown in Figure 1. It belongs to the subgroup of imidazopyridine derivatives. It is formed in higher amounts in model systems and therefore labelling studies are performed more easily. Felton et al (1991a, b) were the first to observe the incorporation of intact phenyl ring from phenylalanine and provided evidence of creatine forming the imidazole moiety of PhIP by observing the isotope label incorporation pattern. However, later studies (Murkovic *et al.*, 1999; Zöchling and Murkovic, 2002) confirmed the involvement of phenylalanine in the mechanism of formation of PhIP through the use of synthetic intermediates and isotopically enriched phenylalanines (^{13}C-2 and ^{13}C-3 labelled). The proposed mechanism is presented in Figure 2. According to this mechanism, phenylacetaldehyde the Strecker aldehyde of phenylalanine, is proposed to be the actual moiety reacting with creatinine as confirmed by the generation of significantly higher levels of PhIP when model systems were spiked with phenylacetaldehyde which then undergoes aldol condensation with creatinine to generate intermediate 1. This intermediate was actually detected in heated meat samples and model systems (Murkovic *et al.*, 1999; Zöchling and Murkovic, 2002). Labelling studies indicated the incorporation of two ^{13}C-2 atoms and one ^{13}C-3 atom in PhIP which did not incorporate any ^{13}C-1 atoms consistant with the proposed pathway. The final steps in PhIP formation was recently elucidated by Zamaro et al. (2014). They proposed the incorporation of ammonia (generated either from creatine or Phe) into the creatinine in intermediate 1 followed by reaction of formaldehyde (generated from either creatine or Phe) with the newly generated amine to form intermediate 2 which undergoes electrocyclic ring closure to form structure 3 followed by oxidation step to finally generate the PhIP.

2.2 A general route to imidazopyridines

Based on the confirmed pathway for the generation of PhIP, a general route to other imidazopyridines can be envisaged as shown in Figure 3. According to the proposed pathway any aldehyde with at least two carbon atoms can undergo aldol condensation with creatinine to form the intermediate 1 (see Figure 3) which in the presence of ammonia or its precursors such as amino acids or creatine can generate intermediate 2 followed by reaction with any other aldehyde to form the imine 3. This imine can undergo electrocyclic ring closure followed by oxidation to generate various imidazopyridine derivatives depending on the nature of the two aldehydes. The presence of more than one type of aldehydes can generate various substituents on the pyridine ring (R and R_1 moieties). All the known members of this group can be envisaged to be formed from appropriate precursors commonly found in meat (Zamaro and Hidalgo, 2015).

2.3 Imidazoquinoline (IQ) and imidazoquinoxaline (IQx) derivatives

There are no proposed or confirmed pathways for the formation of the members of above two subgroups of aminoimidazoazarenes. However, Jägerstad et al (1983) proposed specific precursors such as simple aldehydes, pyrazines or pyridines, creatinine and creatinine/aldehyde aldol adducts. Figure 4 lists a modified version of these precursors to rationalize their incorporation into imidazoquinoline and imidazoquinoxaline derivatives. During thermal treatment of food various heterocyclic ring structures are initially formed at their lower oxidation states such as dihydropyrazine or dihydropyridines shown in Figure 4 and are subsequently undergo aromatization through oxidation or reaction with aldehydes. The initial adducts of dihydropyrazine or dihydropyridines with formaldehyde (structures 1 and 2 in Figure 4) along with structure 1' are proposed as precursors of imidazoquinoline and imidazoquinoxaline derivatives. In this proposal (see Figure 5) structure 1' can undergo aldol-like condensation with 1 to generate structure 2 which itself undergoes vinylogous aldol-condensation to form structure 3 which after dehydration and oxidation can lead to various derivatives of imidazoquinoxalines depending on the nature of the aldehyde reacting with creatinine in the initial step shown in Figure 4. A similar transformation of the precursor 2 shown in Figure 4 with precursor 1' can lead imidazoquinoline derivatives shown in Figure 6.

2.4 Isomeric imidazoquinolines (IQ [4,5-b])

Zamaro and Hidalgo (2015) proposed the interaction of creatinine with benzaldehyde in a similar fashion to that of its interaction with phenylacetaldehyde shown in Figure 2 to generate IQ isomer (IQ[4,5-b]), however, it will be difficult to rationalize a nuceleophilic substitution reaction of the imino group on the creatinine with the aromatic ring which theoretically can undergo only electrophilic aromatic substitution reactions. We can overcome this problem by replacing benzaldehyde with salycilaldehyde as shown in Figure 7, this can allow the formation of a carbonyl functionality on the benzene ring which can now react with nucleophilic nitrogen and at the same time facilitates the conversion of the imine into a reactive amine moiety before the final step of carbonyl amine condensation and formation of IQ isomer.

2.4 β-carbolines

This class of pyrolytic heterocyclic amines can be easily accessed through Pictet-Spengler condensation (Manini et al., 2005; Yaylayan et al., 1990). Tryptophane or tryptamine can form a Schiff base with formaldehyde or acetaldehyde. The Schiff base originating from tryptophane can undergo oxidative decarboxylation to generate harman or norharman moieties after a Pictet-Spengler (P-S) condensation step as shown in Figure 8.

III- PROPOSED PATHWAYS TO POLYCYCLIC AROMATIC HYDROCARBONS (PAH)

Partial oxidation intermediates formed during heating of lipids, fatty acids and carbohydrates are considered the precursors of polycyclic aromatic hydrocarbons (PAH) in meat products. There are no specific and distinct proposed mechanisms or precursors reported in the literature for their formation in food related products. However, a literature review indicated that there could be three possible routes to aromatic ring structure and eventually to PAHs utilizing precursors that are commonly found in food or could be formed under processing conditions. Figure 9 summarizes the proposed pathways. The most efficient and promising pathway among them is the 1,4-cycloaddition reaction between a diene and a dieneophile such as 1,3-butadiene and ethene as elaborated in Figure 10. This reaction is known as Diels-Alder (D-A) reaction which in general can occur efficiently between 100-150^{o}C with good yields, however, the particular interaction between

1,3-butadiene and ethene to generate cyclohexene as a first step in the formation pathway of various PAHs shown in Figure 10 proceeds with 20% yield at 200°C under pressure (Lischka et al., 2004). As shown in Figure 10, cyclohexene can undergo a series of similar D-A reactions with either 1,3-butadiene or ethene followed by partial oxidations and isomerization of the double bonds leading to all possible PAH structures such as anthracene, pyrene, chrysene, etc. Both precursors in this pathway can be generated from high temperature free radical degradation of lipids (Nawar, 1969, 1985) and can undergo Diels-Alder reaction under deep fat frying conditions (Velasco et al., 2008). The other two pathways shown in Figure 9 that can generate aromatic structures from dicarbonyl chemistry through aldol condensation reactions (Guerra and Yaylayan, 2013) or from sugar dehydrations followed by aldol condensation (Haffenden and Yaylayan, 2005) are indirect pathways and require substantial reduction steps before generating PAH moieties.

IV- CONCLUDING REMARKS

In a final remark, recent studies (Samraj et al., 2015) have pointed to a different culprit than the process-induced toxicants discussed above, for the association between red meat consumption and increased cancer risks. The authors in this study, refer to a sialic acid derivative, N-glycolylneuraminic acid (Neu5Gc) found in red meat and its interaction with circulating anti-Neu5Gc antibodies as the root cause for human carcinogenesis rather than the consumption of various thermally generated toxicants. The authors have provided evidence for a new mechanism of carcinogenesis that overrides all other causes including chemical agents generated during processing.

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Table 1. Process specific carcinogens[1]

Curing with nitrites	**Fermentation**	**Smoking**	**Thermal treatments**[2]
N-nitroso compounds	Biogenic amines	3-monochloropropane-1,2-diol (3-MCPD), PAH	PAHs & HAAs

[1]PAH polycyclic aromatic hydrocarbons; HAA heterocyclic aromatic amines
[2]Roasting, grilling, barbecuing, frying, cooking, etc

Figure 1. Classification of heterocyclic aromatic amines (HAAs)

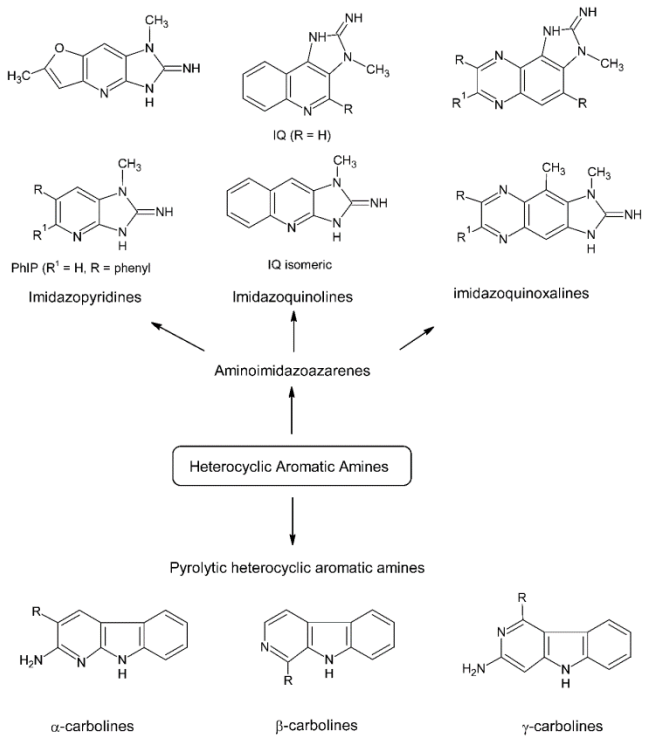


Figure 2. Proposed mechanism of formation of PhIP based on Murkovic, et. al., (1999) and Zamaro et al., (2014). * indicates mixed origin from amino acids or creatine.

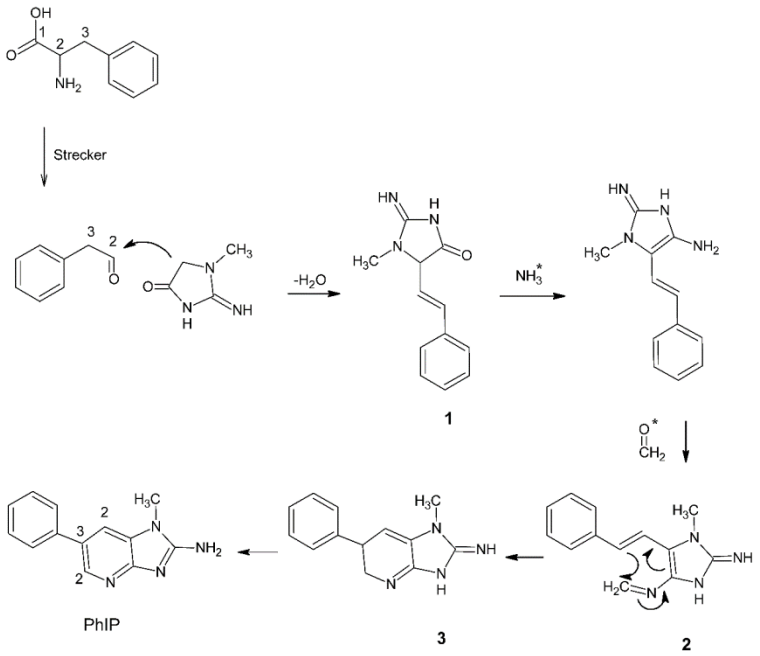


Figure 3. Proposed mechanism of formation of imidazopyridins

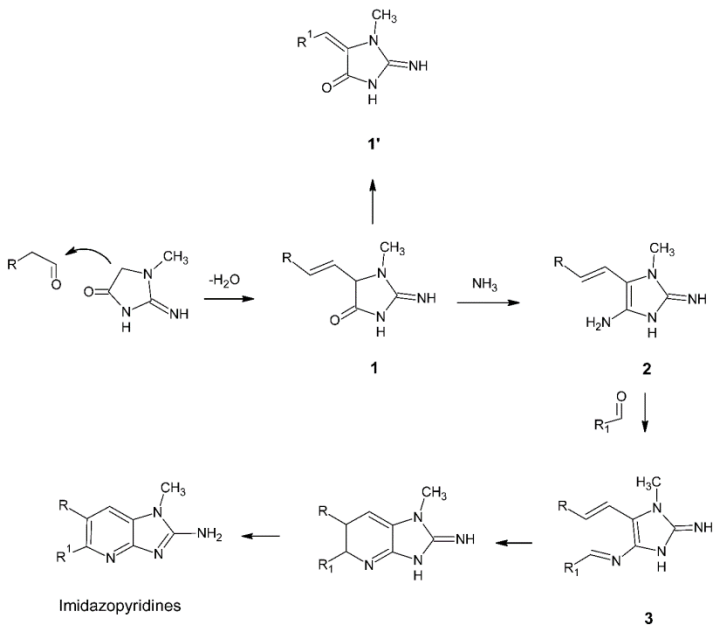


Figure 4. Proposed precursors involved in the formation of imidazoquinoxalines and imidazoquinolines

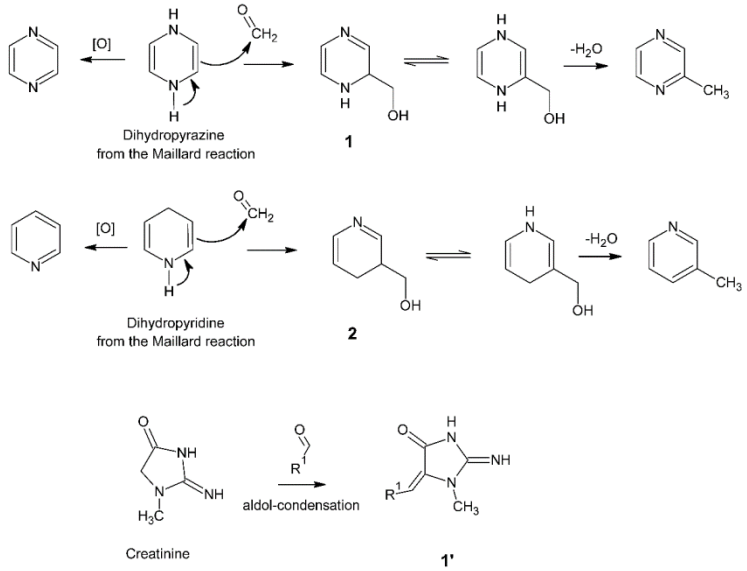


Figure 5. Proposed mechanism of formation of imidazoquinoxalines

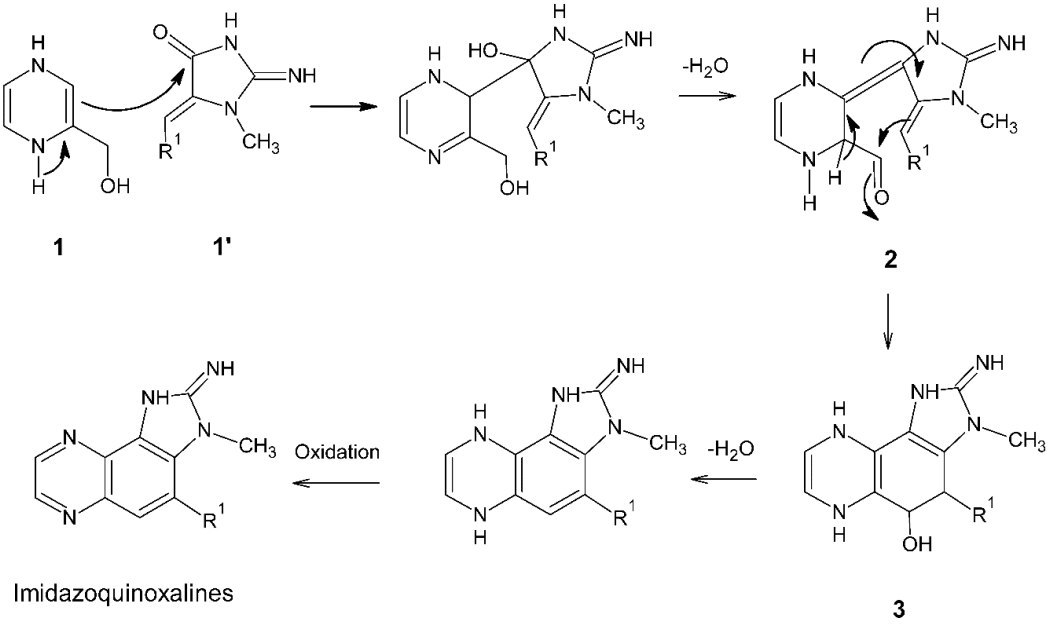


Figure 6. Proposed mechanism of formation of imidazoquinolines

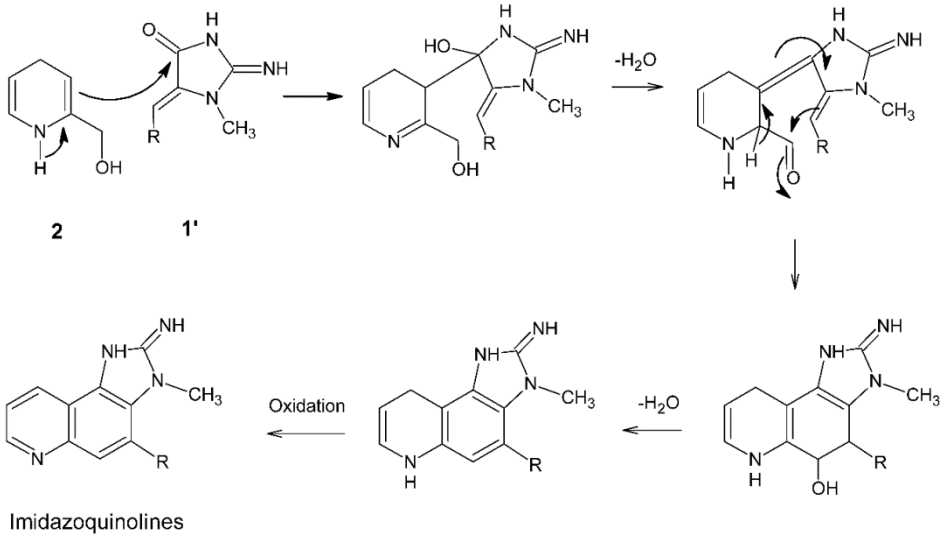


Figure 7. Proposed mechanism of formation of IQ isomer based on Zamaro and Hidalgo (2015)

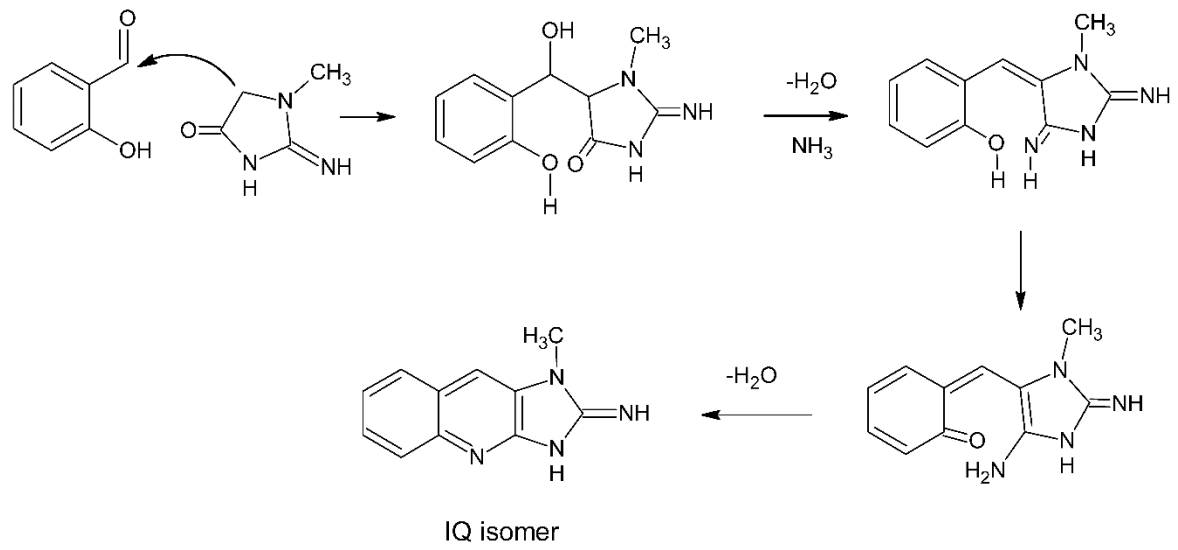


Figure 8. Proposed mechanism of formation of β-carbolines.
P-S = Pictet-Spengler condensation.

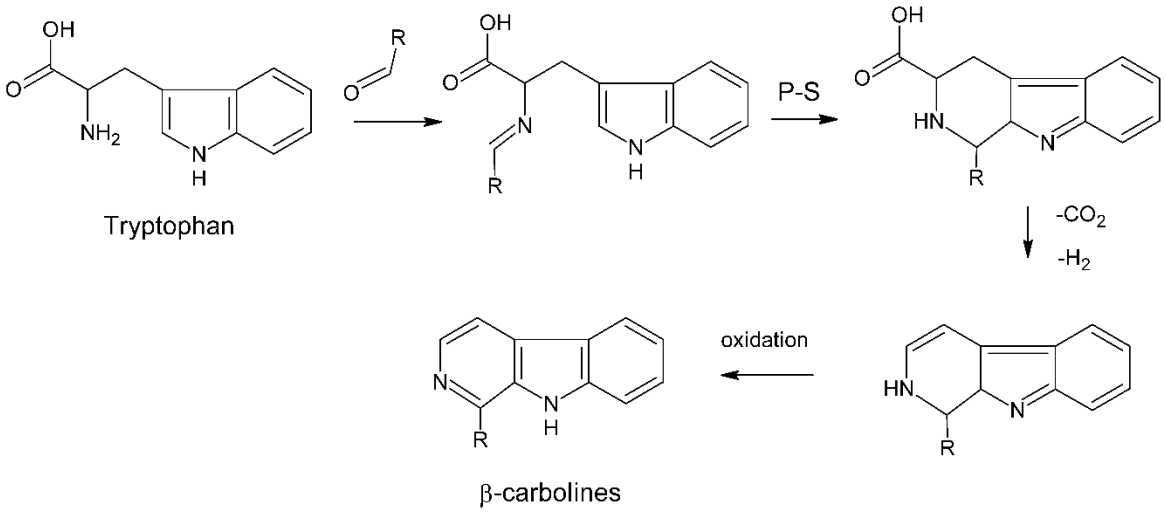


Figure 9. Proposed pathways to polycyclic aromatic hydrocarbons (PAHs).
DA = Diels-Alder reaction

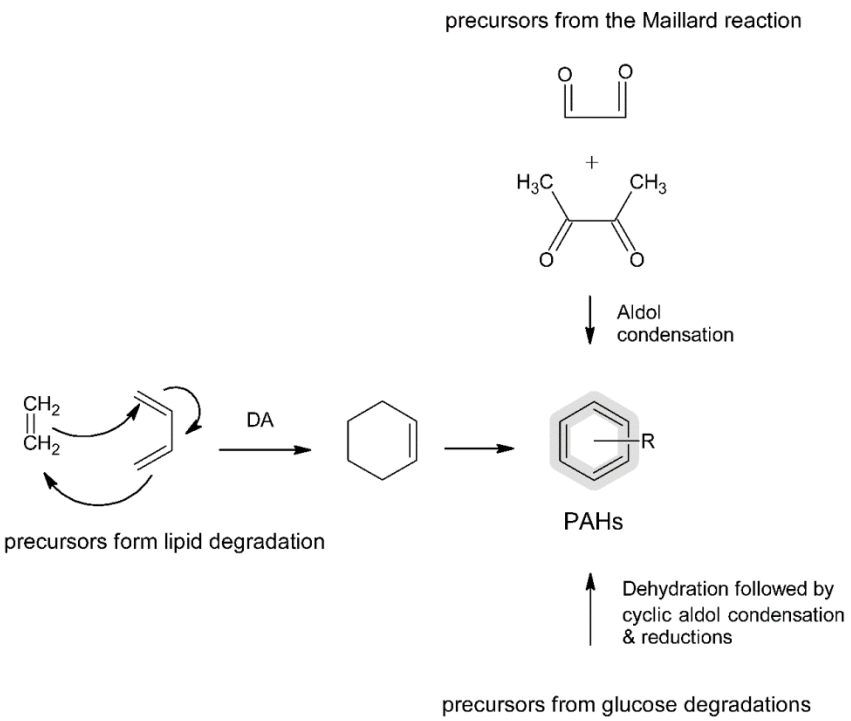
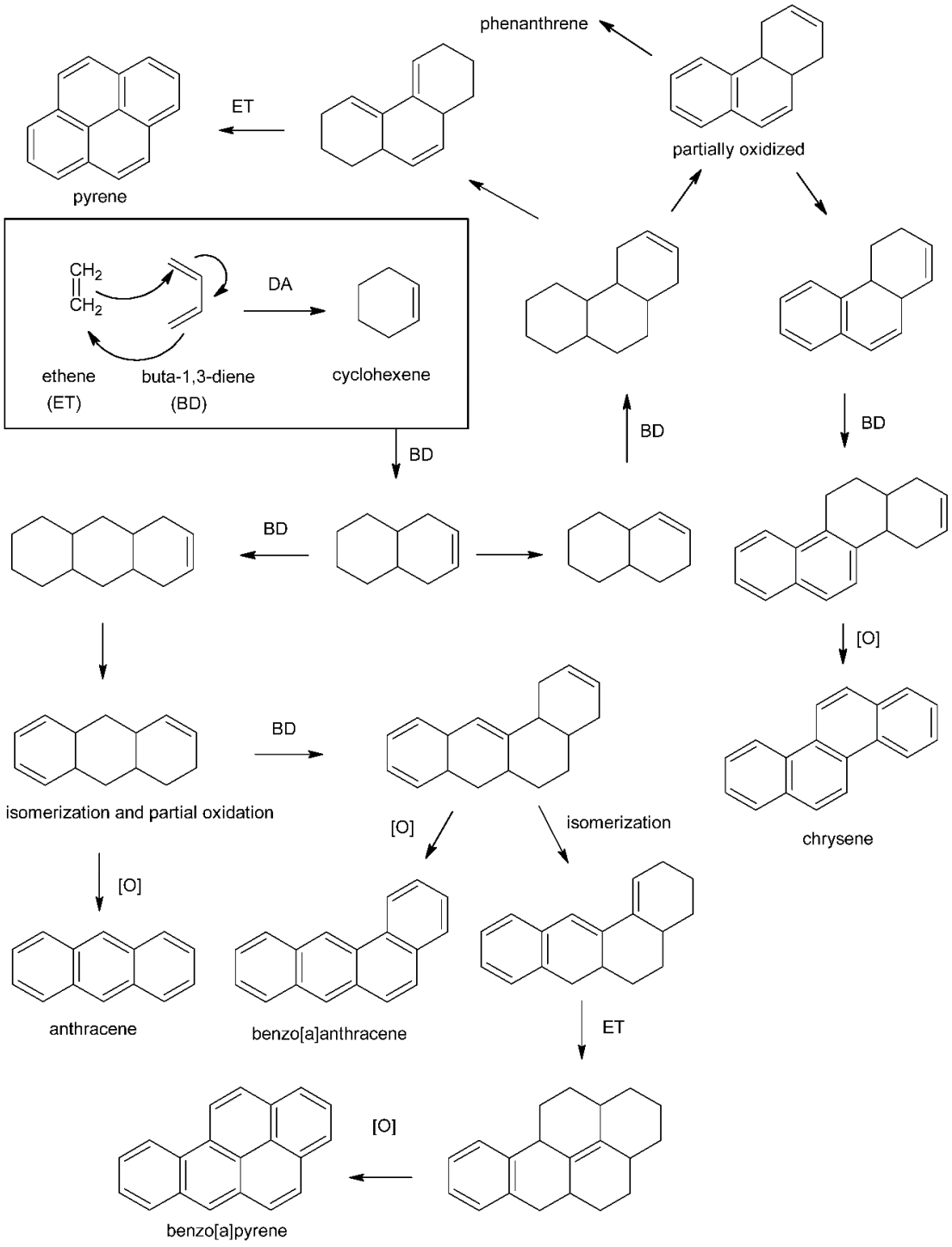


Figure 10. Hypothetical and sequential Diels-Alder (DA) cyclo-condensation reactions of ethene (ET) and buta-1,3-diene (BD) to generate various polycyclic aromatic hydrocarbons (PAHs). (The steps shown involves DA reactions and/or partial oxidations and/or isomerizations of the double bonds).


phenanthrene
partially oxidized
ET
pyrene
DA
CH2
CH2
ethene
(ET)
buta-1,3-diene
(BD)
cyclohexene
BD
BD
BD
BD
[O]
chrysene
isomerization and partial oxidation
BD
[O]
isomerization
[O]
anthracene
benzo[a]anthracene
ET
[O]
benzo[a]pyrene