Synthesis of Metabolites of Polycyclic Aromatic Hydrocarbons

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Abstract: Polycyclic aromatic hydrocarbons (PAHs), regarded as a class of hazardous pollutants due to their persistence and toxicity, are ubiquitous in the environment. Much research has been conducted on aerobic microbial degradation of some PAHs and determination of the degradation pathways for which synthesis of metabolic intermediates and metabolites is required. This mini-review briefly summarizes synthesis of commercially unavailable metabolites of phenanthrene, anthracene, pyrene, fluoranthene, and benzo[a]pyrene.

Key Words: Polycyclic aromatic hydrocarbons, PAH, Microbial degradation, Metabolites, biodegradation, metabolism, catabolism.

I. INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) consist of two or more fused aromatic rings in various structural configurations [1]. PAHs are primarily produced from biogenic, petrogenic, and pyrogenic sources [2]. Biogenic aromatic compounds include aromatic amino acids, lignin compounds and their derivatives which are from biological origins. Petrogenic PAHs are from petroleum crude oils and petroleum-derived products, and are often in abundance of alkyl-substituted PAHs. Pyrogenic PAHs are produced from incomplete combustion of organic materials and are comprised of predominantly unsubstituted PAHs [3]. For example, PAHs in the environment can originate from wood burning heaters, agricultural waste burning, motor vehicle exhaust, cigarette smoke, asphalt road and roofing operations. Dispersed PAHs in the environment accumulate gradually in soil due to their high hydrophobicity and have a high potential for bio-magnification through trophic transfer. They are detected in air [4], soil and sediment [5], surface water, groundwater, and road runoff [6], vegetation [7], and contaminated foods [8]. PAHs are also known to possess potentially deleterious effects on human health.

Hundreds of PAHs have been identified in atmospheric particles, and, however, toxicological endpoint and exposure data are available for 33 PAHs at present, and only 16 are classified as priority pollutants by the U.S. Environment Protection Agency [9]. Bacteria isolated from PAHs-contaminated sites have been employed to study the degradation of PAHs, especially, high molecular weight PAHs in the past several decades. Many PAH metabolites and intermediates are identified and characterized while some are proposed. This mini-review focuses on syntheses of phase I reaction metabolites (i.e., not conjugates) of phenanthrene (**1**), anthracene (**18**), pyrene (**31**), fluoranthene (**45**), and benzo[a]pyrene (**66**) (Fig. **1**). Those metabolites discussed are presently not commercially available, but have been isolated, identified, characterized and reported (Table **1**).

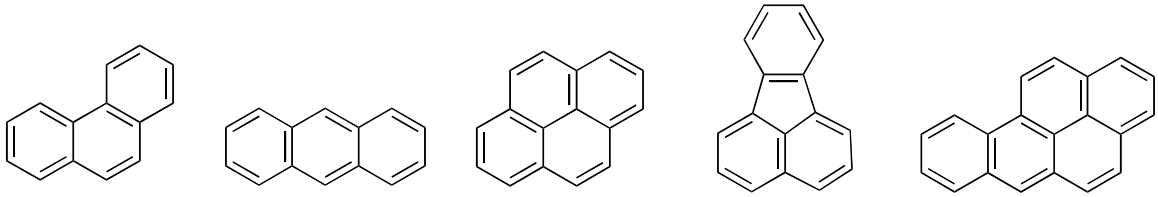

Fig. (1). Structures of phenanthrene (**1**), anthracene (**18**), pyrene (**31**), fluoranthene (**45**), and benzo[a]pyrene (**66**).

It is well known that microbes such as bacteria play a primary role in the degradation of persistent organic pollutants in the environment, including PAHs and heterocyclic aromatic compounds. Many publications have reported aerobic biodegradation of PAHs by various soil bacteria by which PAHs are catabolized into intermediates and metabolites. With identification and characterization of the intermediates and metabolites, degradation pathways of PAHs can be mapped. Such effort is significant to understand the mechanisms of microbial degradation of PAHs and propose viable strategies for cleanup of PAHs-contaminated soil, water, and sediment. However, some important PAH metabolites or metabolic intermediates are often not commercially available. It is helpful for studies of PAH degradation and remediation if syntheses of main PAH metabolites or metabolic intermediates are summarized.

II. MICROBIAL DEGRADATION OF PAHS AND SYNTHESIS OF PAH METABOLITES

1. Microbial Degradation of PAHs

Dioxygenation catalyzed by dioxygenases is a primary initial reaction of PAHs degradation in bacteria and algae while monooxygenation catalyzed by monooxygenases, particularly cytochrome P-450 systems in mammalian cells, occurs mainly in fungi, bacteria, algae as well as animals [10]. PAHs also undergo radical oxidations catalyzed by ligninolytic enzymes such as peroxidaes and laccases, which forms quinones. Dioxygenation produces *cis*-dihydroxydihydro-PAHs while monooxygention forms PAH-epoxides and then *trans*-dihydroxydihydro-PAHs. Subsequently, these PAH-diols and quinones undergo ring cleavages to form hydroxyl and carboxylic-metabolites that are further transformed to primary metabolites. Isolation and identification of microbial degradation products of PAHs have been commonly reported in the literature as summarized in Table **1**. There are two approaches to

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Table 1. Isolated and Identified Aerobic Microbial Metabolites of the Five Representative PAHs Phenanthrene, Anthracene, Pyrene, Fluoranthene and benzo[a]pyrene

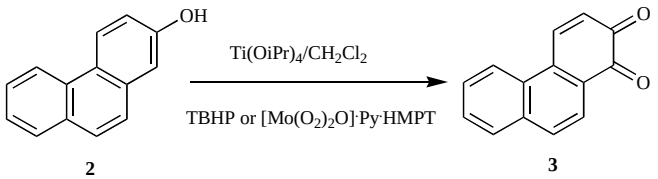
Metabolites		Parent PAHs	Bacteria or Fungi	References	
No.	Chemical Names			Identification	Synthesis
4	*trans*-1,2-Dihydroxy-1,2-dihydrophenanthrene	Phenanthrene	*Cunninghamella elegans*	[49]	[12,13]
6	*cis*-3,4-Dihydroxy-3,4-dihydrophenanthrene		*Mycobacterium* sp. strain 6PY1	[50]	[13]
8	*cis*-9,10-Dihydroxy-9,10-dihydrophenanthrene		*Mycobacterium* sp. strain PYR-1	[51]	[14]
9	1,2-Dihydroxy-phenanthrene		*Mycobacterium vanbaalenii* PYR-1	[52]	[15]
11	9,10-Dihydroxy phenanthrene		*Mycobacterium* sp. strain PYR-1	[51]	[16,17]
14	1,2-Dimethoxy phenanthrene		*Mycobacterium vanbaalenii* PYR-1	[52]	[28,19]
17	2H-benzo[h]chromen-2-one		*Sphingomonas* sp. strain P2	[53]	[20]
21	*trans*-1,2-Dihydroxy-1,2-dihydroanthracene	Anthracene	*Rhodococcus* sp.	[54]	[13,15]
22	1,2-Dihydroxyanthracene		*Mycobacterium* sp. strain LB501T	[55]	[15]
26	9,10-Dihydroxy-9,10-dihydroanthracene		*Mycobacterium* sp. strain PYR-1	[51]	[22,23]
27	9,10-Dihydroxyanthracene		*Mycobacterium* sp. strain PYR-1	[51]	[22,24]
30	6,7-Benzocoumarin		*pseudomonads*	[56]	[20]
32	*cis*-4,5-Dihydroxy-4,5-dihydropyrene	Pyrene	*Mycobacterium* sp.	[57]	[26]
34	*trans*-4,5-Dihydroxy-4,5-dihydropyrene		*Mycobacterium* sp.	[57]	[26]
35	4,5-Dihydroxypyrene		*Mycobacterium* sp.	[57]	[27]
40	Methylphenanthrene-4-carboxylate		*Mycobacterium vanbaalenii* PYR-1	[58]	[28]
44	*trans, trans*-1,3-Butadiene-1,2,4-tricarboxylic acid		*Mycobacterium vanbaalenii* PYR-1	[58]	[29]
47	*trans*-2,3-Dihydroxy-2,3-dihydrofluoranthene	Fluoranthene	*Mycobacterium* sp. strain PYR-1	[60]	[39]
49	2-Hydroxyfluoranthene		*Sphingomonas* sp. LB126	[61]	[40]
53	1-Hydroxyfluoranthene		*Sphingomonas* sp. LB126	[61]	[41]
58	3-Hydroxyfluoranthene		*Sphingomonas* sp. LB126	[61]	[42,43]
63	8-Hydroxyfluoranthene		*Sphingomonas* sp. LB126	[61]	[41,44]
65	7-Hydroxyfluoranthene		*Sphingomonas* sp. LB126	[61]	[41]
67	*cis*-4,5-Dihydroxy-4,5-dihydrobenzo[a]pyrene	Benzo[a]pyrene	A mutant strain of *Beijerinckia*	[62]	[46]
74	*trans*-7,8-Dihydroxy-7,8-dihydrobenzo[a]pyrene		A mutant strain of *Beijerinckia*	[62]	[13,47]
78	1-Hydroxypyrene-2-carbaldehyde		*Sphingomonas yanoikuyae* JAR02	[45]	[48]
85	2-Hydroxypyrene-1-carbaldehyde		*Sphingomonas yanoikuyae* JAR02	[45]	[48b]

isolate, identify and characterize the metabolites. One is to chemically synthesize them in laboratories after the structures of the metabolites are proposed. Another approach is biosynthesis of the metabolites by microbial cultivations followed by isolation and characterization of the metabolites. The metabolites either chemically or biochemically synthesized or commercially purchased are then used to identify the metabolites in a metabolic pathway. It is noteworthy that separate cultivations of a specific metabolite are often necessary in order to construct metabolic pathways.

2. Phenanthrene Metabolites

Phenanthrene (**1**) is commonly found in soils, estuarine waters and sediments, and other terrestrial and aquatic sites. Although **1** is not mutagenic or carcinogenic, it has been shown to be toxic to marine diatoms, gastropods, mussels, crustaceans, and fish. It is the smallest PAH to have a "bay-region" and a "K-region", and, therefore, it is often used as a model substrate for studies on metabolism of carcinogenic PAHs [11].

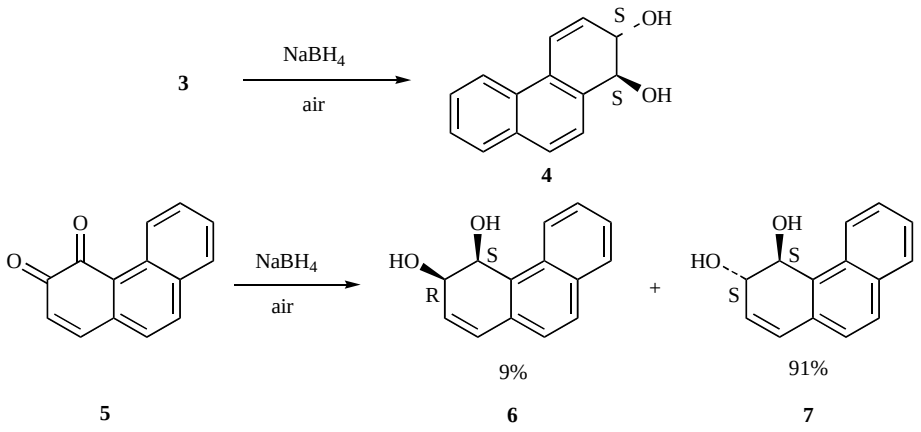
Phenanthrene-1,2-dione (**3**) was prepared in several routes with the starting material of 1-hydroxyphenanthrene [12a], 2-hydroxyphenanthrene (**2**) [12b], 1-amino-2-hydroxyphenanthrene [12c] or 1,2-dihydroxy-1,2,3,4-tetrahydrophenanthrene [12d]. Among them, the synthetic method reported by Krohn *et al.* in 1989 [12b] is more practical with higher yield 56% (Scheme **1**). Compound **2** was treated with transition-metal complex including titanium isopropoxide [$Ti(OiPr)_4$], vanadyl acetylacetonate [$VO(acac)_2$] in dry dichloromethane at -23 ºC under nitrogen atmosphere, and then oxidized by *tert*-butylhydroperoxide (TBHP), or Mimoun oxodiperoxo molybdenum complex {$[Mo(O_2)_2O]\cdot PyHMPT$}. Upon the completion of the reaction, the mixture was acidified with 10% sulfuric acid to obtain **3**.



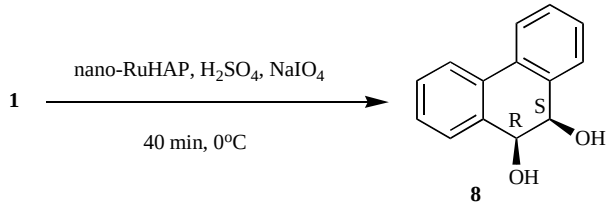
2 **3**

Scheme 1. Synthesis of phenanthrene-1,2-dione from 2-hydroxyphenanthrene.

Non-K-region dihydro diols of PAH play an important role in the metabolism of PAHs. Platt and Oesch [13] found that **3** was reduced with sodium borohydride ($NaBH_4$) in 87% ethanol in the presence of oxygen to form *trans*-1,2-dihydroxy-1,2-dihydrophenanthrene (**4**) in 65% yield. When the isomer phenanthrene-3,4-dione (**5**) of **3** underwent the same reaction, a mixture of 9% *cis*-3,4-dihydroxy-3,4-dihydrophenanthrene (**6**) and 91% *trans*-3,4-



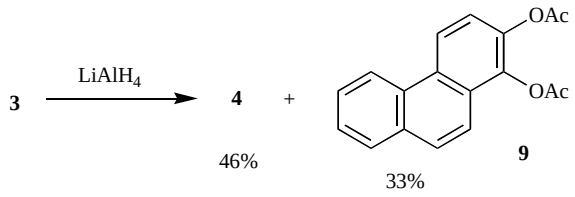
Scheme 2. Synthesis of *trans*-1,2-dihydroxy-1,2-dihydrophenanthrene (**4**), *cis*-3,4-dihydroxy-3,4-dihydrophenanthrene (**6**), and *trans*-3,4-dihydroxy-3,4-dihydrophenanthrene (**7**).



Scheme 3. Synthesis of *cis*-9,10-dihydroxy-9,10-dihydrophenanthrene from **1**.

dihydroxy-3,4-dihydrophenanthrene (**7**) were formed as detected by thin layer chromatography and high performance liquid chromatography (HPLC). Compound **7** was obtained in 55% yield with preparative HPLC (Scheme **2**). To prepare a K-region dihydro diol of **1**, Che *et al.* [14] reported that **1** was cis-dihydroxylated directly to form *cis*-9,10-dihydroxy-9,10-dihydrophenanthrene (**8**) in 60% yield catalyzed by a novel kind of catalyst ruthenium nanoparticles supported on hydroxyapatie (nano-RuHAP) in the presence of $NaIO_4$ and H_2SO_4 in a mixture of ethyl acetate (EtOAc), acetonitrile (MeCN) and water (H_2O) (3:3:1 v/v/v) (Scheme **3**).

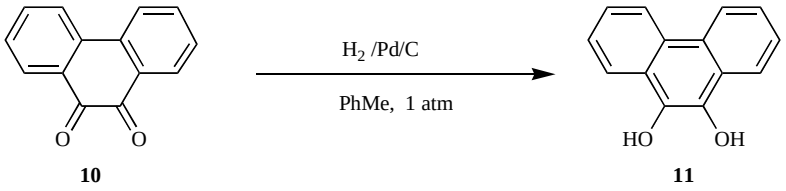
1,2-Dihydroxyphenanthrene (**9**), one of aromatization metabolites after dihydroxylation, can be prepared through reduction of **3** with lithium aluminum hydride ($LiAlH_4$), accompanying formation of **4**. **9** was isolated as its diacetate form in a yield of 33% [15] (Scheme **4**).



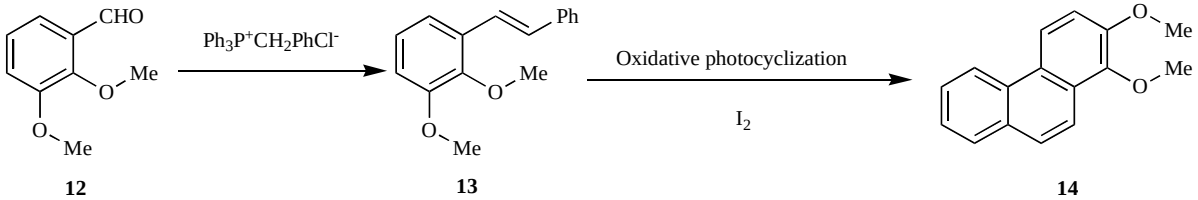
Scheme 4. Synthesis of 1,2-dihydroxyphenanthrene (diacetate) from **3**.

Reduction of phenanthrene-9,10-dione (**10**) with Zn powder in boiling acetic acid (AcOH) gives 9,10-dihydroxyphenanthrene (**11**) [16], which, however, **11** can be converted easily to **10** when exposed to air during drying. In 2004, Guido *et al.* [17] reduced **10** with hydrogen gas in the presence of Pd/C in toluene under atmospheric pressure to afford **11** in 75% yield, and anhydrous **11** can be stored under air for a long period of time (Scheme **5**).

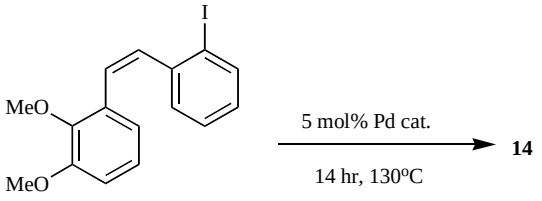
Methylated forms of PAH-diols and PAH-dihydrodiols are often needed for the identification and analysis on gas chromatography (GC) or GC-mass spectrometry (GC-MS). 1,2-Dihydroxyphenanthrene can be methylated to yield 1,2-dimethoxyphenanthrene (**14**). In 1989, Mallory *et al.* [18] described that 2,3-dimethoxybenzaldehyde (**12**) was coupled with benzyltriphenylphosphonium chloride ($Ph_3P^+CH_2PhCl^-$) to give 1-(2,3-dimethoxystyryl)benzene (**13**) in a yield of 83% via a Wittig reaction followed by oxidative photocyclization in cyclohexane in the presence of I_2 to obtain **14** in yield 63% (Scheme **6**). In 2007, Kamikawa *et al.* [19] prepared **14**



Scheme 5. Synthesis of 9,10-dihydroxyphenanthrene from phenanthrene-9,10-dione.



Scheme 6. Synthesis of 1,2-dimethoxyphenanthrene from 2,3-dimethoxybenzaldehyde.



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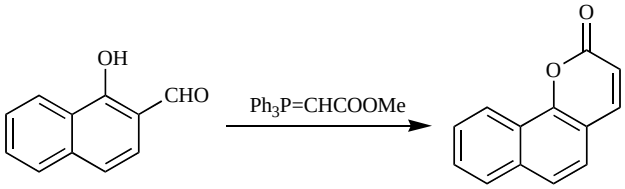
Scheme 7. Synthesis of 1,2-dimethoxyphenanthrene from iodostilbene.



16 **17**

Scheme 8. Synthesis of 7,8-benzocoumarin from 1-hydroxynaphthalene-2-carbaldehyde.

from 1-(2,3-dimethoxystyryl)-2-iodobenzene (**15**) in yield 99% utilizing a palladium (Pd)-catalyzed aromatization reaction (Scheme **7**).

7,8-Benzocoumarin (**17**) is a naphthalene-based lactone intermediate formed from the cleavage of hydroxylated three fused rings. Keum *et al.* [20] in 2005 reported a preparation method of **17** from 1-hydroxynaphthalene-2-carbaldehyde (**16**) via a Wittig reaction (Scheme **8**).

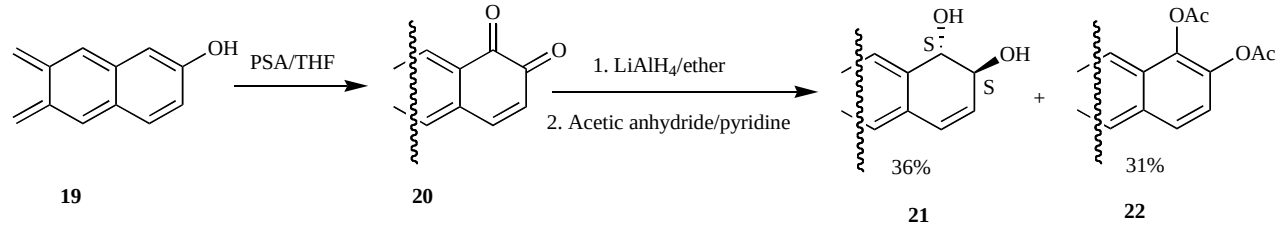
3. Anthracene Metabolites

Being a PAH member with three-fused aromatic rings in linear, anthracene (**18**) is found in high amounts in PAH-contaminated environments. Although it is not genotoxic or carcinogenic, it does represent a threat to the environment due to its toxicity to aquatic life, particularly its photo-induced toxicity [21].

Although a non-ortho dihydroxylation occurs at an initial stage of degradation to produce 9,10-dihydroxy-9,10-dihydroanthracene (**26**) due to its characteristic structure, degradation of **18** resembles that of **1** to form some similar structures of metabolic intermediates or metabolites. *trans*-1,2-Dihydroxy-1,2-dihydroanthracene (**21**) and 9,10-dihydroxy-9,10-dihydroanthracene (**26**) are two representative metabolic intermediates after **18** undergoes initial dihydroxylation. Sukumaran and Harvey [15] oxidized 2-hydroxyanthracene (**19**) with access phenylseleninic anhydride (PSA) in the presence of 50% NaH in anhydrous tetrahydrofuran (THF) to provide anthracene-1,2-dione (**20**) in 54% yield. Compound **20** was reduced with $LiAlH_4$ in anhydrous ethyl ether to afford **21** in 36% yield. The inorganic residue after ethyl ether extraction was treated with glacial acetic acid in THF and was extracted with ethyl ether again. The residue was acetylated by acetic anhydride to obtain 1,2-dihydroxyanthracene diacetate (**22**) in 31% yield after the organic phase was dried and condensed (Scheme **9**). In 1983, Platt and Oesch [13] improved the yield to 72% in a reduction reaction of **20** to **21** with $NaBH_4$.

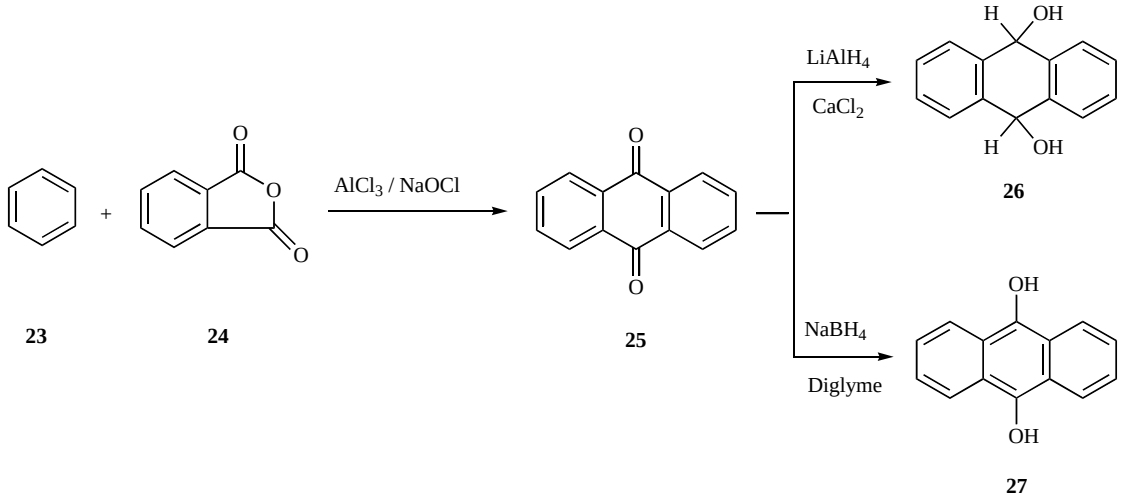
Condensation of benzene (**23**) with phthalic anhydride (**24**) afforded anthracene-9,10-dione (**25**) [22] that was further reduced to result in **26** by $LiAlH_4$ in the presence of calcium chloride ($CaCl_2$) [23] and 9,10-dihydroxyanthracene (**27**) [24] by $NaBH_4$ in diglyme (Scheme **10**).

6,7-Benzocoumarin (**30**), a ring cleavage intermediate after dihydroxylation of **18**, was synthesized by Keum *et al.* [20a]. (3-Methoxynaphthalen-2-yl)methanol (**28**) was oxidized by pyridinium chlorochromate (PCC) in the presence of sodium acetate [20b] to form 3-methoxynaphthalene-2-carbaldehyde (**29**) that was treated with methyl triphenylphosphoranylidene acetate



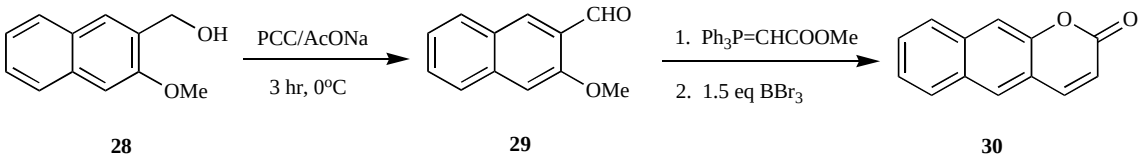
19 **20** **21** **22**

Scheme 9. Synthesis of *trans*-1,2-dihydroxy-1,2-dihydroanthracene and 1,2-dihydroxyanthracene (diacetate).



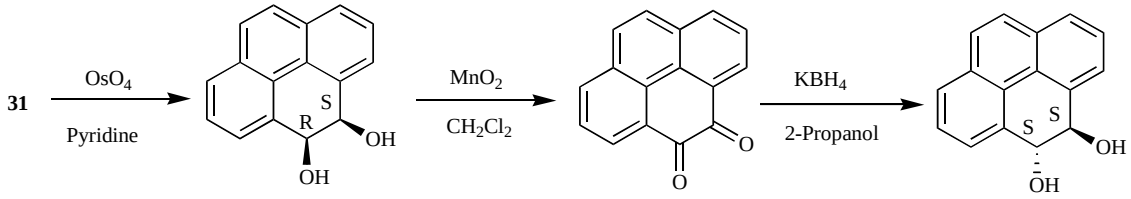
23 **24** **25** **26** **27**

Scheme 10. Synthesis of anthracene-9,10-dione, 9,10-dihydroxy-9,10-dihydroanthracene and 9,10-dihydroxyanthracene from benzene and phthalic anhydride.



28 **29** **30**

Scheme 11. Synthesis of 6,7-benzocoumarin from (3-methoxynaphthalen-2-yl)methanol.



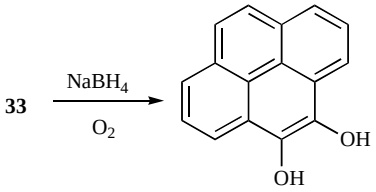
32 **33** **34**

Scheme 12. Synthesis of *cis*-4,5-dihydroxy-4,5-dihydropyrene, pyrene-4,5-dione and *trans*-4,5-dihydroxy-4,5-dihydropyrene from pyrene.

($Ph_3P=CHCO_2CH_3$) followed by a boron-mediated dealkylative ring closure to provide **30** via a Wittig reaction (Scheme **11**).

4. Pyrene Metabolites

Pyrene (**31**) is a 4-rings-fused PAH. The structural symmetry and stability enable **30** recalcitrant to be biologically attacked. Studies [25] revealed that microbial degradation of **31** begins with *ortho*-dihydroxylation, and then undergoes various biological reactions including aromatization, ring cleavage, and etherification, and so on to form small molecular metabolites such as phthalic acid, benzyl acetic acid and benzyl propenoic acid. Among many pyrene metabolites, only a few of them are commercially unavailable.



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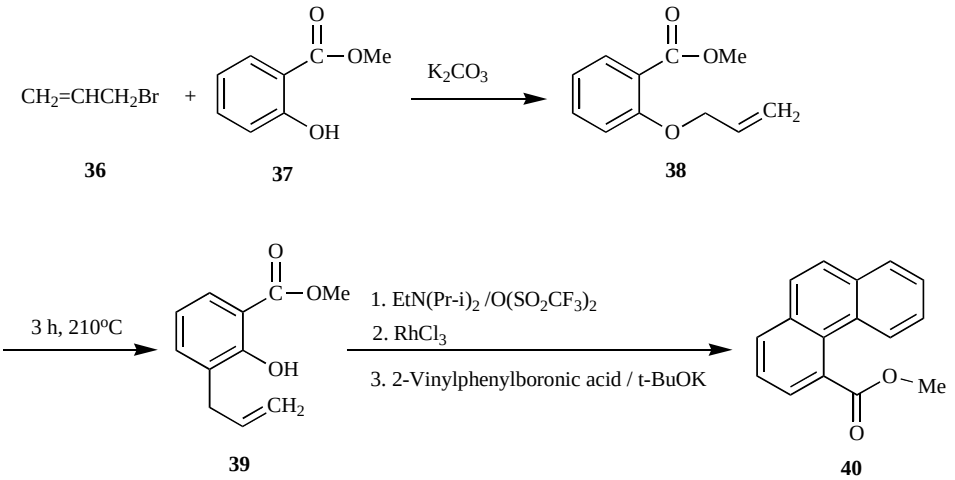
Scheme 13. Synthesis of 4,5-dihydroxypyrene from pyrene-4,5-dione.

Oxidation reaction of **31** by osmium tetraoxide (OsO_4) in pyridine afforded *cis*-4,5-dihydroxy-4,5-dihydropyrene (**32**) followed by further oxidation with active manganese dioxide (MnO_2) in dichloromethane to generate pyrene-4,5-dione (**33**) (Scheme **12**). Compound **33** was reduced with KBH_4 in 2-propanol to give *trans*-4,5-dihydroxy-4,5-dihydropyrene (**34**). It is noteworthy that **34** can be converted easily back to **33** via autoxidation. Compound **34** needs to be stored at 0 ºC [26] under nitrogen atmosphere. In addition, **33** was reduced with $NaBH_4$ in the presence of oxygen to provide non optically active 4,5-dihydroxypyrene (**35**) [27] (Scheme **13**).

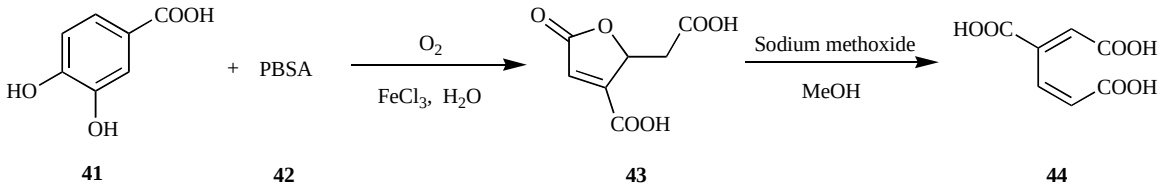
Methylphenanthrene-4-carboxylate (**40**) is a representative metabolic intermediate after ring cleavage of **31**. Barrett *et al.* [28] reported that allyl bromide (**36**) was condensed with salicylic acid methyl ester (**37**) through etherification to afford methyl 2-(allyloxy)benzoate (**38**). Compound **38** was transformed to methyl 3-allyl-2-hydroxybenzoate (**39**) via thermal Claisen rearrangement followed by Suzuki coupling reaction with 2-vinylphenylboronic acid to give **40** (Scheme **14**).

trans,trans-1,3-Butadiene-1,2,4-tricarboxylic acid (**44**) was synthesized through oxidation of 3,4-dihydroxybenzoic acid (**41**) by oxygen in a buffer solution containing $FeCl_3$ and (2-pyridylmethyl-) bis((4-sulfo-2-pyridyl)methyl)amine (PBSA) (**42**) followed by treatment with sodium methoxide in dry methanol [29] (Scheme **15**).



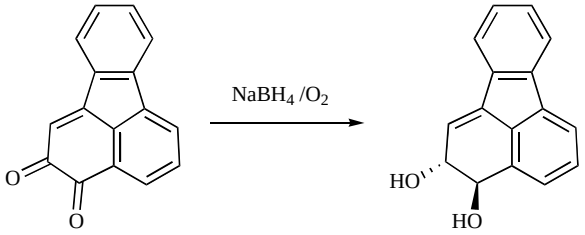
36 **37** **38** **39** **40**

Scheme 14. Synthesis of methylphenanthrene-4-carboxylate from allyl bromide and methyl 2-hydroxybenzoate.



41 **42** **43** **44**

Scheme 15. Synthesis of *trans, trans*-1,3-butadiene-1,2,4-tricarboxylic acid from 3,4-dihydroxybenzoic acid.



46 **47**

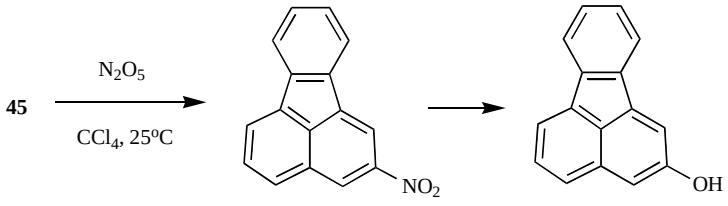
Scheme 16. Synthesis of *trans*-2,3-dihydroxy-2,3-dihydrofluoranthene from fluoranthene-2,3-dione.

5. Fluoranthene Metabolites

Fluoranthene (**45**) is a member of the non-alternant PAH class that contains a five-membered ring condensed with naphthalene and benzene rings. Human exposure to fluoranthene is from inhalation of particulates in air, tobacco smoke, and ingestion from contaminated food and water [30]. Compound **45** not only is genotoxic [31], but also can potentiate the carcinogenicity of benzo[a]pyrene [32]. For example, acute and subchronic exposure of **45** through oral administration to laboratory animals affects several toxicological endpoints that include decreased body weight, increased liver weight, and decreased blood chemistry tests. Compound **45** is also capable of eliciting neurobehavioral toxicity [33], suppressing the immune system [34], affecting human lung airway anion transport [35], and inhibiting the photosynthetic processes in pea plants and lichens [36]. Therefore, **45** has been used as a model compound for biodegradation studies of structurally related PAHs such as fluorene, acenaphthylene, carbazole, dibenzothiophene, dibenzofuran, and dibenzodioxin [37]. Studies of PAH-contaminated soil revealed that a number of bacterial isolates can degrade fluoranthene, especially bacteria in the genera *Rhodococcus* and *Mycobacterium* [38]. A few tens of metabolic intermediates and metabolites were identified by various techniques, and most are listed in commercial catalogs. Synthetic routes of those commercially unavailable are summarized below.

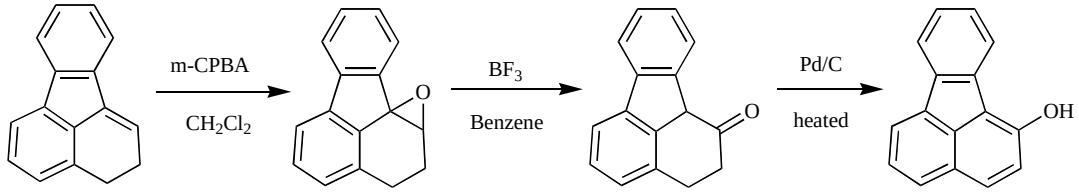
trans-2,3-Dihydroxy-2,3-dihydrofluoranthene (**47**), one of the metabolites from the initial dihydroxylation of **45**, was prepared according to a method [39] similar to the preparation of **4** (Scheme **16**).

Several mono hydroxyl **45** isomers were found. These regioisomers require different synthetic routes. For example, **45** was nitrated by nitrogen pentoxide (N_2O_5) to form 2-nitrofluoranthene (**48**) followed by hydroxylation to afford 2-hydroxyfluoranthene (**49**) [40] (Scheme **17**). 2,3-Dihydrofluoranthene (**50**) was oxidized with *m*-chloroperoxybenzoic acid (m-CPBA) in dichloromethane followed by isomerization catalyzed by trifluoroborane (BF_3) in dry benzene, and then reduction and aromatization in the presence of 10% Pd/C in refluxing xylene to form 1-hydroxyfluoranthene (**53**) [41] (Scheme **18**). 4-Amino-1-naphthol (**54**) was treated with isoamyl nitrite in ethanol to form 4-diazonaphthalen-1(4H)-one (**55**) [42], which was condensed with anthranilic acid (**56**) in the presence of isopentyl nitrite in dichloromethane followed by a thermal rearrangement in *o*-dichlorobenzene to afford 3-hydroxyfluoranthene **58** [43] (Scheme **19**). 8-Dihydroxyfluoranthene (**63**) was prepared in four steps. Acenaphthylene (**59**) reacted first with buta-1,3-diene via a Diels-Alder reaction to form 6b,7,10,10a-tetrahydrofluoranthene (**60**) followed by reduction with diborane in THF (or $NaBH_4$ in the presence of acetic acid in THF at 0 °C [44]) to afford 8-hydroxy-6b,7,8,9,10,10a-hexahydrofluoranthene (**61**) in a yield 100%. Compound **61** was oxidized with pyridinium chlorochromate (PCC) to give **62** that was dehydrogenated by refluxing



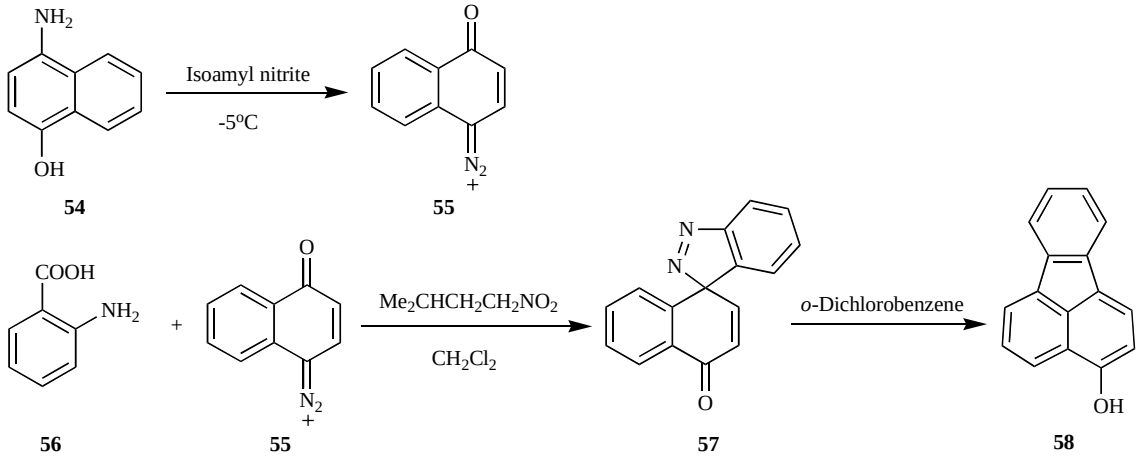
48 **49**

Scheme 17. Synthesis of 2-hydroxyfluoranthene from fluoranthene.

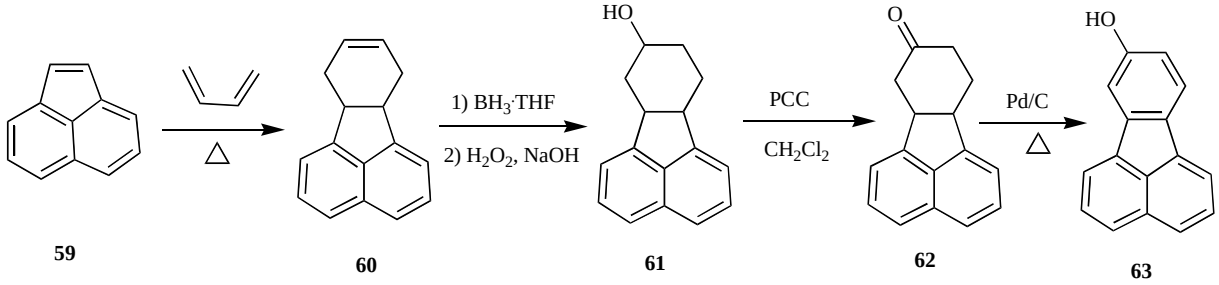


50 **51** **52** **53**

Scheme 18. Synthesis of 1-hydroxyfluoranthene from 2,3-dihydrofluoranthene.

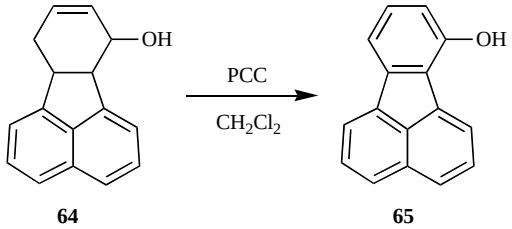


Scheme 19. Synthesis of 3-hydroxyfluoranthene from 4-amino-1-naphthol.



Scheme 20. Synthesis of 8-hydroxyfluoranthene from acenaphthylene.

with Pd/C under nitrogen to provide **63** [41] (Scheme **20**). Differently, 7-hydroxy-6b,7,10,10a-tetrahydrofluoranthene (**64**) was dehydrogenated by reacting with pyridinium chlorochromate (PCC) in dichloromethane at 20 ºC under nitrogen to form 7-hydroxyfluoranthene (**65**) [41] (Scheme **21**).



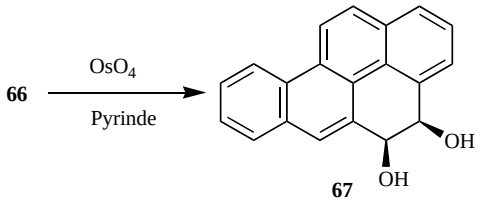
Scheme 21. Synthesis of 7-hydroxyfluoranthene from 7-hydroxy-6b,7,10, 10a-tetrahydrofluoranthene.

6. Benzo[a]pyrene Metabolites

Benzo[a]pyrene (**66**), a representative high molecular weight PAH, is of environmental concern due to its known carcinogenicity and bioaccumulation potential. Few studies described microbial degradation of **66**. Its metabolites include several dihydrodiols and one ring-cleavage product 10-oxabenzo[def]chrysene-9-one [45].

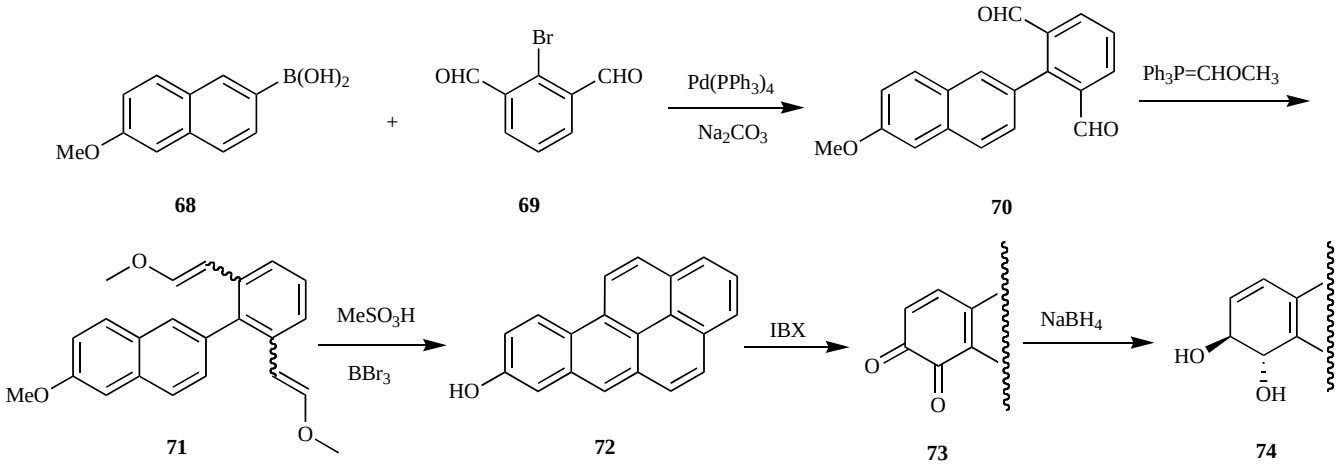
cis-4,5-Dihydroxy-4,5-dihydrobenzo[a]pyrene (**67**), as one of dihydroxylated metabolites of **66,** was prepared by direct oxidation of **66** by OsO_4 in pyridine [46] (Scheme **22**), like the preparation of **31**.

Another dihydroxylated metabolite, *trans*-7,8-dihydroxy-7,8-dihydrobenzo[a]pyrene (**74**), was synthesized in five steps. First, Suzuki coupling of 6-methoxynaphthaleneboronic acid (**68**) and 2-bromobenzene-1,3-dialdehyde (**69**) formed 2-(2-methoxynaphthalen-6-yl)benzene-1,3-dialdehyde (**70**), which **70** was readily transformed to 2-(2,6-bis(methoxyvinyl)phenyl)-2-methoxynaphthalene (**71**) via the Wittig reaction. Compound **71** was transformed to 8-hydroxybenzo[pqr]tetraphene (**72**) via methanesulfonic acid-catalyzed cyclization and demethylation of boron tribromide. A noteworthy hypervalent iodine reagent *o*-iodoxybenzoic acid (IBX) was then used to oxidize **72** to benzo[a]tetraphene-7,8-dione (**73**). Compared with the methods reported by Sukumaran and Harvey [15] in 1980 ($(PhSeO)_2O$ as oxidant, yield 42%) and Krohn *et al*. [12] in 1989 ($[Mo(O_2)_2O]$PyHMPT as oxidant, yield 56%), IBX for oxidation of the PAH *β*-phenols to form PAH *o*-quinones provides an excellent overall yield of 92%. Finally, **73** was reduced to **74** with $NaBH_4$ in ethanol in the presence of oxygen [13, 47] (Scheme **23**).

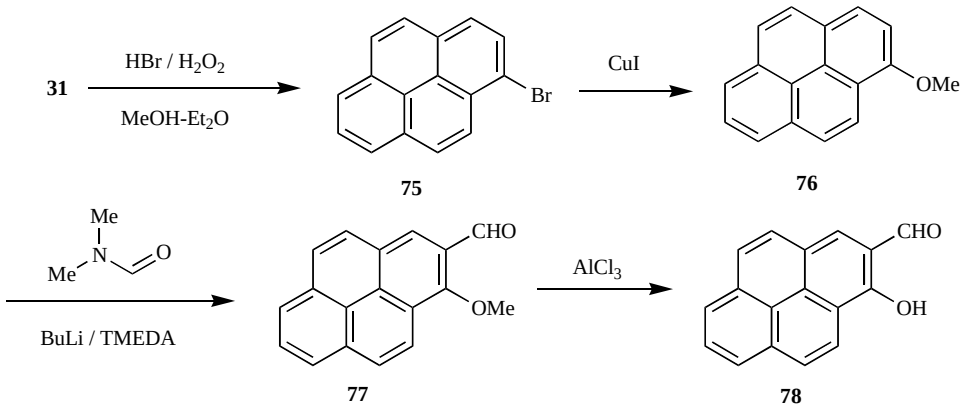


Scheme 22. Synthesis of *cis*-4,5-dihydroxy-4,5-dihydrobenzo[a]pyrene from benzo[a]pyrene.

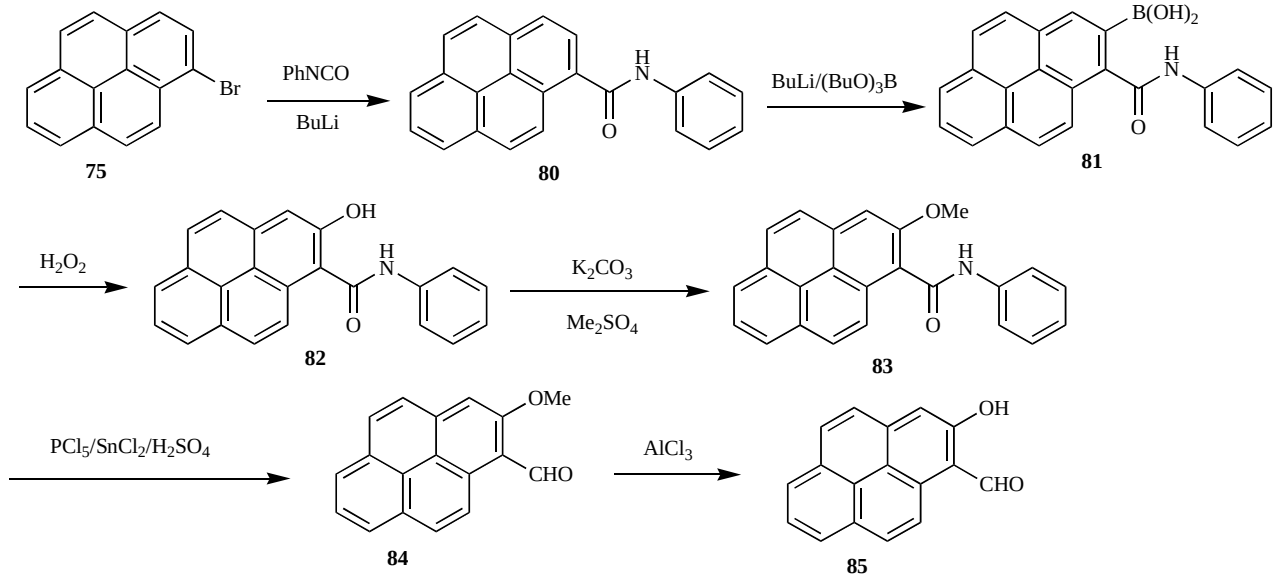
Two key metabolites of **66** from ring cleavage after dihydroxylation are 1-hydroxypyrene-2-carbaldehyde (**78**) and 2-hydroxypyrene-1-carbaldehyde (**85**). The reactant pyrene (**31**) was treated with hydrobromic acid (HBr) and hydrogen peroxide (H_2O_2) to form 1-bromopyrene (**75**) [48a] followed by etherification of sodium methoxide (CH_3ONa) in the presence of CuI to obtain 1-methoxypyrene (**76**). Compound **76** was formylated with N,N-dimethylformamide and tetramethylethylenediamine (TMEDA) to



Scheme 23. Synthesis of *trans*-7,8-dihydroxy-7,8-dihydrobenzo[a]pyrene from 6-methoxynaphthaleneboronic acid and 2-bromobenzene-1,3-dialdehyde.



Scheme 24. Synthesis of 1-hydroxypyrene-2-carbaldehyde from pyrene.



Scheme 25. Synthesis of 2-hydroxypyrene-1-carbaldehyde from 1-bromopyrene.

afford 1-methoxypyrene-2-carbaldehyde (**77**) that was then cleaved with $AlCl_3$ in dichloromethane to obtain **78** [48b] (Scheme **24**). Compound **85**, an isomer of **78,** was prepared from **75** via a different approach. Compound **75** was condensed with phenyl isocyanate (PhNCO) (**79**) to form *N*-phenylpyrene-1-carboxamide (**80**) followed by reaction with tributyl borate [$(BuO)_3B$] in the presence of butyl lithium (BuLi) to give 1-(phenylcarbamoyl)pyren-2-yl-2-boronic acid (**81**). The intermediate **81** was oxidized with H_2O_2 and then methylated with dimethyl sulfate to form 2-methoxy-N-phenylpyrene-1-carboxamide (**83**). After that, oxidation of **83** with

phosphorus pentachloride (PCl_5) formed 2-methoxypyrene-1-carbaldehyde (**84**) that was then reacted with $AlCl_3$ to produce **85** [48b] (Scheme **25**).

III. CONCLUSION

Bioaccumulation, persistence and toxicity of PAHs cause concerns over their occurrence and fate in the environment and their exposure to humans and wildlife. It is known that aerobic microbial degradation of PAHs start with dioxygenations, monooxygenations and radical oxidations. Understanding of metabolic pathways will provide significant insights into the mechanisms and network of biodegradation. Availability of PAH metabolite standards is required for elucidation of biodegradation pathways of PAHs. To date, although many PAH metabolites are commercially available, less stable, structurally complicated ones have been yet synthesized. This review summarized synthesis approaches of important, known metabolites of five un-substituted PAHs that have been used commonly as model chemicals in microbial degradation studies. The information described here is hopefully helpful for research in microbial degradation and advances of bioremediation technologies for cleanup of persistent organic pollutants in the environment. In particularly, PAH metabolites may be bioactive, stimuli to trigger biological events. Availability of PAH metabolites may help omics studies such as metabolomics and proteomics of PAH biodegradation.

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